# Sensitivity profiling of genes responsible for multi-/extensively drug resistant mutations in Mycobacterium tuberculosis isolated from the Tshepong National Health Laboratory Service Referral Tuberculosis Laboratory in North-West

**by**

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# DECLARATION OF INDEPENDENT WORK

I, MOTSHIDISI NTANJANE JEANETT, student number _____, do hereby declare that this research project submitted to the Central University of Technology, Free State for the degree of Master of Health Sciences in Biomedical Technology, is my own independent work, and complies with the Code of Academic Integrity, as well as other relevant policies, procedures, rules and regulations of the Central University of Technology, Free State, and has not been submitted before to any institution by myself or any other person in fulfilment (or partial fulfilment ) of the requirements for the attainment of a qualification.

**Student Signature:** **Date: 23/09/2025**

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# ABSTRACT

**Background:** Tuberculosis (TB), an infection that is caused by *Mycobacterium tuberculosis* organisms, remains one of the leading infections causing morbidity and mortality globally, with the emergence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains, causing a major public-health challenge. Tuberculosis is transmitted through the inhalation of airborne droplets expelled by a person with active TB when they cough or sneeze. Several diagnostic methods are used in the diagnosis of tuberculosis, including microscopy, culture, and molecular assays. The first-line drugs used in the treatment of TB are rifampicin, isoniazid, pyrazinamide, and ethambutol. The second-line drugs used in the treatment of multidrug-resistant tuberculosis (MDR-TB) include fluoroquinolones (such as levofloxacin and moxifloxacin), second-line injectable agents (such as amikacin, kanamycin, and capreomycin), and oral agents, including ethionamide, prothionamide, cycloserine, and para-aminosalicylic acid (PAS).

**Aim:** To determine sensitivity profiling and detect the genes responsible for MDR/XDR mutations in tuberculosis isolated from the Tshepong National Health Laboratory Service (NHLS) TB laboratory from May 2023 to March 2025.

**Methods**: Retrospective data were retrieved from the National Health Laboratory service through the Academic Affairs and Research Management Systems. The data were for the patients infected by *Mycobacterium tuberculosis* at Tshepong TB Referral Laboratory in North-West. The data were analysed using Microsoft 365 Excel, Descriptive statistics, Chi-square, Fisher's exact test, and ANOVA. The results are presented in tables and figures.

**The results:** A total of 340 Mycobacterium tuberculosis-positive isolates from the Tshepong NHLS TB Referral Laboratory in the North-West Province were analysed. The largest proportion of cases was observed in the 31–40-year age group (30%), reflecting the high burden of disease in the economically active population. MDR-TB represented the most frequent classification, accounting for 62.1% of all cases, indicating a substantial burden of multidrug resistance within the study population.

Among the 340 specimens evaluated, 64.4% (n = 219) were resistant to rifampicin, while 21% (n = 70) were sensitive and 15% (n = 52) produced unsuccessful results. The highest levels of susceptibility were observed for amikacin (98.8%), capreomycin (98.8%), kanamycin (98.8%), and fluoroquinolones (93.2%), confirming the continued effectiveness of these second-line drugs. Ethionamide exhibited the highest resistance rate (12.7%).

Molecular analysis revealed that the *rpoB* gene, associated with rifampicin resistance, was the most frequently affected locus (n = 219; 64.4%). The highest mean melting temperature was recorded for the *inhA* promoter gene (75 °C), followed by *gyrA* (72 °C) and *rrs* (71 °C). The lowest mean melting temperature (68 °C) was observed for the *eis* promoter gene associated with kanamycin resistance.

Facility-level analysis demonstrated that Tshepong Hospital reported the highest number of TB cases (n = 155; 45.6%), followed by Moses Kotane Hospital (n = 45; 13.2%) and other small facilities (n = 56; 16.5%). The highest number of MDR-TB cases was also recorded at Tshepong (n = 95; 61.3%), followed by Moses Kotane (n = 32; 71.3%). Together, Tshepong and Moses Kotane Hospitals accounted for most MDR-TB cases in the cohort. XDR-TB cases were reported at Tshepong (3.2%), Moses Kotane (6.7%), other small facilities (1.8%), and the NIC Bodenstein Hospital (16.67%), indicating the presence of extensively drug-resistant TB in multiple facilities, although at low prevalence.

**Conclusion:** These findings highlight the need for enhanced molecular diagnostic capacity and consistent monitoring of resistance trends in routine clinical practice. Targeted interventions at high-risk facilities should be prioritised to prevent further transmission. Future research should investigate the genetic diversity of circulating strains and evaluate the effectiveness of tailored treatment approaches for drug-resistant TB.

**Keywords:** MDR-TB, XDR-TB, melting temperature, North-West

# LIST OF ABBREVIATIONS

MTBC – Mycobacterium *tuberculosis complex*

TB – Tuberculosis

MDR – Multidrug-Resistant

XDR – Extensively Drug Resistant

DSTB – Drug-Susceptible Tuberculosis

UNC – Unclassified

H -TB – Isoniazid-resistant Tuberculosis

WHO – World Health Organization

PANTA – Polymyxin-B, ampholericin-B, nalidixic acid, trimethoprim, azilocillin

PCR – Polymerase Chain Reaction

DOTS – Directly Observed Treatment, Short - course

TST – Tuberculin skin test

LPA – Line Probe Assay

DNA – Deoxyribose Nucleic Acid

INH – Isoniazid

RIF – Rifampicin

PZA – Pyrazinamide

EMB – Ethambutol

CFZ – Clofazimine

FLQs – Fluoroquinolones

BDQ – Bedaquiline

RR/MDR – Rifampicin-Resistant/Multidrug-resistant

CIF – Ciprofloxacin

MFX – Moxifloxacin

LFX – Levofloxacin

OFX – Ofloxacin

NHLS – National Health Laboratory Service

CDW – Central Data Warehouse

DRC – Democratic Republic of Congo

HIV – Human Immunodeficiency Virus

DST – Drug Susceptibility Testing

AARMS – Academic Affairs and Research Management System

HSREC – Health Science Research Ethics Committee

POPIA – Protection of Personal Information Act

SOP – Standard Operating Procedure

NALC-NaOH – N-Acetyl-L-Cysteine-Sodium Hydroxide

MGIT – Mycobacterial Growth Indicator Tube

AFB – Acid Fast Bacilli

NTM – Non-tuberculosis mycobacteria

MOTT – Mycobacteria Other Than Tuberculosis

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# APPENDICES

# CHAPTER 1: INTRODUCTION

The Mycobacteriaceace family encompasses a diverse group of bacteria that show different traits of pathogenicity in animals and humans and exhibit various hosts' reservoirs and growth dynamics in culture (Kanabalan *et al*., 2021).The Mycobacterium tuberculosis complex (MTBC) is a group of mycobacterium that comprises of *M Mycobacterium tuberculosis (M.tuberculosis), Mycobacterium africanum (M.afrinanum) Mycobacterium bovis (M.bovis), Mycobacterium canettii (M.canetti, Mycobacterium microti (M.macroti), Mycobacterium pinnipedii (M.pinnipedii) and Mycobacterium caprea (M.caprae)* that are related genetically (Kanabalan *et al*., 2021). Tuberculosis (TB) caused by the *Mycobacterium tuberculosis (M.tb*) bacteria is an important public-health issue around the world, being a chronic infectious disease (Rasool, 2019). Drug-resistant bacterial infections are on the rise globally, which has placed a sharp focus on drug-resistant tuberculosis (TB), its diagnosis and treatment (Mirzayez *et al.,* 2021). Multidrug-resistant (MDR)-TB is caused by *Mycobacterium tuberculosis* strains that are resistant to at least isoniazid and rifampicin, two first-line drugs used to treat TB (Mirzayez *et al.,* 2021). In early 2021, the World Health Organization (WHO) recommended new definitions of extensively drug-resistant (XDR) and pre-XDR tuberculosis (TB) (Dahl *et al.,* 2024). Previously, pre-XDR TB was informally defined as TB caused by *M.tb* strains with resistance to rifampin and isoniazid-plus resistance to either a fluoroquinolone or a second-line injectable, but not both (Dahl *et al.,* 2024). Now, pre-XDR TB is officially defined as strains with resistance to rifampin, isoniazid, and a fluoroquinolone (levofloxacin or moxifloxacin), whereas XDR TB is now defined as additional resistance to ≥1 group A drug (bedaquiline or linezolid), replacing the second-line injectables used in the former definitions (Dahl *et al*., 2024).

Tuberculosis is transmitted primarily through sneezing and coughing and is considered among the oldest diseases recognized by mankind (Rasool, 2019). It affects mainly the lungs (pulmonary TB) but can also affect other organs or tissues of the body (extrapulmonary TB) (Rasool, 2019). According to Moule and Cirillo (2020), active tuberculosis usually presents as a pulmonary infection consisting of a cough lasting longer

than a few weeks, often associated with the production of blood sputum and a myriad of other classic symptoms including chills, fever, weakness unintentional weight loss and night sweats. Latent tuberculosis generally does not produce any clinical symptoms and patients may never know that they have been infected unless reactivation occurs (Moule and Cirillo, 2020).

According to Gill *et al. (*2021), improving the efficiency and accuracy of a TB diagnosis contributes to treatment efficiency. Several diagnostics techniques, including microscopy, culture and molecular probes are used to evaluate the presence of mycobacteria in respiratory specimens, where each technique has its advantages and disadvantages (Srivastava *et al*., 2020). A culture of *Mtb* in a suitable medium remains the gold-standard diagnostics test (Gill *et.al.,* 2021). Culture-based methods are extremely sensitive, but susceptible to contamination problems and therefore, subjected to decontamination steps prior to inoculation of agar or liquid culture media, which add inaccuracy as some mycobacteria die during the specimen processing (Srivastava *et al.,* 2020). The NALC-NaoH-based specimen decontamination method is used the most (Srivastava *et al*., 2020). A cocktail of antibiotics, PANTA (polymyxin-Amphotericin-B, nalidixic acid, trimethoprim, azilocillin) is used to kill other bacteria to promote mycobacterial growth (Srivastava *et al.,* 2020). The major benefit of the advent of liquid-based systems is the rapid time to detection, often reducing time to growth by half, with a mean time to detection of 12.8 days, compared to 25.1–25.5 days for the previously mentioned solid media (Gill *et al*., 2021). GeneXpert MTB/RIF is an automated polymerase chain reaction (PCR) test used to diagnose Active TB(ATB) rapidly and detect rifampicin resistance (Nogueira *et al.,* 2022). Unlike conventional nucleic acid amplification tests, it amplifies and detects PCR in about 2 hours using automated assays, thus requiring minimal training and bio-safety measures (Nogueira *et.al*., 2022). According to Wobudeya *et al. (*2022), smear microscopy is the oldest microbiology test for TB. Simple, fast and inexpensive, it has been the cornerstone of the WHO DOTS (Directly Observed Treatment, Short-course) TB control strategy, relying on the direct visualization of the acid-fast bacilli, using conventional microscopy based on Ziehl-Neelsen staining of fluorochrome staining with standard/light-emitting diodes (LED) fluorescence microscopy (Wobudeya *et al*., 2022).

Sputum smear microscopy is important for the diagnosis of tuberculosis, because it identifies patients with active tuberculosis who feed the chain of disease transmission (Silva *et al*., 2021). Fluorescence microscopy can increase the capacity to detect mycobacteria by 10%, compared with conventional light microscope (Silva *et al.,* 2021). An increase in the sensitivity of smear microscopy can also be activated by using sputum centrifugation or sedimentation (Silva *et al*., 2021).

According to Ludi *et al*. (2023), it has been shown that antibiotics against purified cord factor antigen, trehalose-6,6'-dimycolate (TOM), the most abundant cell-wall component of *Mtb* bacilli, can be used for rapid serodiagnosis of pulmonary TB. A chest X-ray is used to evaluate patients with suspected intraocular TB, since the lungs are most often the primary site of TB infection (Ludi *et al*., 2023). Chest-computed tomography and positron-emission tomography scans are not routinely performed due to high costs, even though superior delineation between concomitant parenchymal hilar or pleural lesions in normal or inconclusive chest X rays (Ludi *et al*., 2023). To overcome the limitations of the Tuberculin Skin Test (TST), several new skin tests and interferon-gamma release assays (IGRAs) have been developed (Huang *et al*., 2022). These include the Dia skin test, C-Tb skin test, EC-Test, and T-cell spot of the TB assay, as well as QuantiFERON-TB Gold In-Tube, QuantiFERON-TB Gold-Plus, LIAISON QuantiFERON-TB Gold Plus test, and LIOferon TB/LTBI(Huang *et al*., 2022). The basis of the LPA is that the pre-labelled amplification product is captured by the DNA probe solidified on the membrane strip and detected by colorimetry, and the results of LPA appear as a linear band (Huang *et al.*, 2022). LPA can detect drug resistance to first-line TB drugs INH and RIF, and there are different version of commercial products, including Geno Type MTBDR plus 1.0 (Hain Life Science) and INNO-LPA Rif TB kit (Innogenetic) (Huang *et al.,* 2022). Rapid identification assays capable of distinguishing between *Mtb* Complex and NTM after positive cultures are the basis for initiating early anti-B therapy (Huang *et al.*, 2022).

According to Saderi *et al*. (2022), improving treatment success will require access to rapid diagnostic tests able to detect and classify drug resistance reliably and inform clinicians designing individual regimens. Among the most-used medicines in the treatment of TB, isoniazid (INH), pyrazinamide (PZA), ethambutol (EMB), and rifampicin (RIF) are called first-line drugs (Rossini and Dias, 2023). These drugs are the first choice of treatment for

TB, which has a regimen of about six months with co-administration of all of them in the first four months and two of them in the last two months (Rossini and Dias, 2023). The updated treatment guidelines recommend the inclusion of CFZ (Clofazimine) as a core component of DR-TB regimens, highlighting its efficacy and manageable safety profile in combination with other Group A drugs such as FQs (Fluoroquinolones) and BDQ (Bedaquiline) (Nasiri *et al.,* 2025). Another important change in the management of TB was the recommendation of all-oral as well as shorter regimens for DR-TB (Nasiri *et.al.,* 2025). The WHO currently recommends two shorter regimens for RR/MDR-B – a fixed 6-month regimen and a 9–12-month regimen for eligible patients in addition to the longer regimens of 18–20 months (Nasiri *et al.,* 2025). Furthermore, according to the 2023 update of The Working Group on New TB Drugs, which tracks the global TB pipeline, there are more than 19 promising compounds in different stages of clinical development, as well as various combinations of existing, repurposed, and new agents, currently investigated in phase 2 or 3 clinical trials as components of potential new treatment regimens (Nasiri *et al.,* 2025)

With time, the mutations accumulated within the *Mtb* genome and specifically in the proteins serving as drug targets, have caused the development of resistance against various first- and second-line anti-TB drugs and further its self-transformation into pre-extensively drug-resistant (pre-XDR), multi-drug-resistant (MDR) and extensively drug-resistant (Singh, 2021). The drug resistance-associated genes *rpoB, inhA, KatG, pncA*, *gry A* and gry *B* are known to confer resistance against rifampicin (RIF), isoniazid (INH), pyrazinamide (PZA) and fluoroquinolone (FLQ), ciprofloxacin (CIF), moxifloxacin (MFX), levofloxacin(LFX) and ofloxacin (OFX), respectively (Singh, 2021). This is a retrospective study that will focus on the sensitivity profiling of genes responsible for multidrug-resistant and extensively drug-resistant mutations in *Mtb* isolated from the Tshepong National Health Laboratory Service (NHLS) Referral Tuberculosis (TB) Laboratory in the North-West Province.

## 1.1 Research problem

MDR and XDR tuberculosis TB remain major public-health concerns due to resistance to both first- and second-line anti-TB drugs. The literature shows that studies on drug

resistance profiling and genetic mutations have been conducted in other countries, as well as in South Africa and certain provinces. However, no such study has been undertaken at the Tshepong Referral TB Laboratory, despite it being the central facility for TB culture testing in the North-West Province. The laboratory processes approximately 80% of all TB specimens in the province and receives samples from multiple facilities, including Gelukspan, Moses Kotane, Mafikeng, Rustenburg, and surrounding clinics in Klerksdorp. This makes Tshepong a critical site for monitoring TB drug-resistance patterns.

Despite its central role, there are limited data on the sensitivity profiles of *Mycobacterium tuberculosis* isolates tested at Tshepong and the genetic mutations responsible for resistance. Furthermore, demographic and epidemiological patterns such as age distribution and regional burden remain inadequately documented. This lack of evidence limits the ability to evaluate treatment outcomes, monitor trends in MDR- and XDR-TB, and determine whether the burden of drug-resistant TB is increasing or decreasing across the province. Given that the North-West Province is divided into two major regions – East and Bophirima – understanding both regional and facility level variations in TB resistance is essential for guiding public-health interventions and strengthening TB control programs.

## 1.2 The aim of the study

The aim of this study was to determine sensitivity profiling and detect genes responsible for MDR/XDR mutations in tuberculosis isolates from the Tshepong National Health Laboratory Service (NHLS) TB laboratory.

## 1.3 Objectives

- To determine the Baseline Characteristics of *M. tuberculosis* isolates based on the age group and TB classification.
- To determine TB prevalence in the North- West Province based on facility.
- To determine the sensitivity profiles of first-line anti-tuberculosis drugs in *Mycobacterium tuberculosis*-positive specimens using data obtained from the NHLS Central Data Warehouse (CDW).

- To assess the sensitivity profiles of second-line anti-tuberculosis drugs in specimens demonstrating resistance to first-line drugs, based on data from the CDW.
- To identify genetic mutations associated with drug resistance in *Mycobacterium tuberculosis* isolates based on their Melting Peak temperature, utilizing data retrieved from the CDW.
- To identify prevalence of TB cases, MDX and XDR TB according to facility distribution.

## 1.4 The hypothesis

- A significant proportion of *Mycobacterium tuberculosis*-positive specimens are resistant to at least one first-line anti-tuberculosis drug.
- Specimens resistant to first-line drugs are more likely to demonstrate resistance to second-line drugs, contributing to MDR and XDR TB cases.
- Drug resistance in *Mycobacterium tuberculosis* is significantly associated with specific genetic mutations detectable through molecular diagnostic data.
- The prevalence of drug-resistant TB differs significantly across age groups, with certain age categories being more affected than others.
- The burden of drug-resistant TB is unevenly distributed across regions and healthcare facilities in the North-West Province, with certain areas/facilities showing higher case cases.

# CHAPTER 2: LITERATURE REVIEW

## 2.1 Background

Geographically, in 2021, the majority of people who developed TB were located in the WHO South-East Asia region (45%) followed by the WHO African region (23%) and the WHO Western Pacific region (18%) (Alsayed and Gunosewoyo, 2023). Four countries accounted for more than half of the global TB burden: the two WHO South-East Asian countries, India (28%) and Indonesia (9.2%) in addition to the two WHO Western Pacific countries, China (7,4%) and the Philippines (7.0%) (Alsayed and Gunosewoyo, 2023). Three lists of high burden countries (HBCs) have been defined by the WHO for TB, TB/HIV and multidrug-resistant TB (MDR-TB), respectively, for the first five years of ENDTB strategy (2016–2020), wherein at least 48 countries appear on the list (Singh *et al.,* 2019). The 14 countries present in all three include Angola, China, the Democratic Republic of Congo, Ethiopia, India, Indonesia, Kenya, Mozambique, Myanmar, Nigeria, Papua New Guinea, South Africa, Thailand and Zimbabwe (Singh *et al.,* 2019). These HBCs annually account for 84% of global TB, 83% of TB/HIVand 87% of MDR-TB cases, respectively (Singh *et al.,* 2019). TB low-burden countries are the least affected by TB and annually report a very low incidence of active TB disease that is less than 10 cases per 100 000 populations (Singh *et al*., 2019). These are represented by the high-income group of countries such as Canada, USA, Australia, Western Europe and New Zealand (Singh *et al.,* 2019).

The Democratic Republic of the Congo (DRC), home to an estimated 81 million people, is one of 14 countries on the World Health Organization (WHO) list of countries with high TB, TB/human immunodeficiency virus (HIV), and MDR-TB burdens (Bisimwa *et al*., 2021). In 2018, the estimated TB incidence rate was 322 per 100 000 with 60 000 TB-related deaths (Bisimwa *et al.,*2021). The estimated prevalence of MDR/RIF-resistant TB in the DRC was 1.7% and 9.5% in new and previously treated TB cases, respectively, but the accuracy of these estimates is limited by low laboratory coverage in many areas for the performance of MTB culture and drug susceptibility testing (DST) (Bisimwa *et al.,* 2021). According to the 2020 WHO global report, Ethiopia was among the high MDR-TB burden countries, with an estimated incidence of MDR/RR TB in 0.71% of new cases and

12% of previously treated cases in 2019 (Worku *et al.,* 2022). The majority of TB drug-resistance studies were conducted in only a few areas of the country and did not include marginalized pastoral areas such as the Somali region (Worku *et al.,* 2022). Nonetheless, the less-developed health infrastructure in pastoralist communities and the poor compliance with treatment due to patients' nomadic lifestyles contribute to the occurrence of MDR-TB in the Somali region (Worku *et al*., 2022).

## 2.2 Trends and Transmission of MDR and XDR-TB

According to Nandlal *et al.* (2022), over 1.4 million people died from TB in 2020, with more than 95% of these deaths occurring in low- and middle-income countries. Despite a 20% decrease in TB mortality from 2015 to 2020, the prevalence rates of MDR-TB and XDR-TB continue to rise among both new and previously treated TB cases (Nandlal *et al*., 2022). Recent data suggest that person-to-person transmission of MDR- and XDR-TB is now the driving force of the global DR-TB burden although acquired drug resistance continues to contribute to the evolution of drug resistance in the context of TB treatment mismanagement (Nandlal *et al*., 2022).

The combined effects of low fertility and high life expectancy have accelerated global population ageing. As the backbone of socioeconomic development, adolescents and adults aged 15–49 years are of special importance (Kunping *et al*., 2025). As they are in their reproductive and prime working years, they experience many physical, emotional and psychosocial changes during the transition to adulthood (Kunping *et al*., 2025). Diseases, career development, higher education, interpersonal relationships, and family formation can all influence these changes (Kunping *et al.,* 2025). Also, this age group is uniquely positioned in DR-TB transmission chains, with potential DR-TB spread in schools, workplaces, etc. (Kunping *et al.,* 2025). Therefore, focusing on the burden and trends of DR-TB in adolescents and adults holds important value (Kunping *et al*., 2025).

## 2.3 Genetic Mechanisms of Drug Resistance in TB

According to Traore *et al*. (2023), numerous previous studies identified various genes that encode anti-TB drug targets and briefly discussed various mechanisms of resistance to

RIF and INH (Traore *et al.,* 2023). More than 95% of RIF resistance is associated with *rpoB* gene alterations in an 81-bp area (Traore *et al*., 2023). INH resistance appears to be more complex and has been linked to numerous genes, most notably *katG* and the *inhA promoter* region (Traore *et al.,* 2023).MDR TB, defined as TB resistant to both rifampin and isoniazid, are a global public-health threat (Kherabi *et al.,* 2022). In 2019, there were 465 000 incidence cases of RR TB, among of which 78% were MDR TB (Kherabi *et al*., 2022). In addition, an estimated 3.6% of new TB cases and 18% of previously treated TB cases have developed MDR-TB in 2021 (Diriba *et al*., 2023). Moreover, on average, in 2019, 6.2% of XDR were estimated among patients treated for MDR-TB (Diriba *et al.,* 2023). Globally, 16.2% of RR/MDR TB isolates have acquired resistance to fluoroquinolones, indicating the need for an update in resistance definitions (Kherabi *et al.,* 2022). Thus, in January 2021, the WHO defined pre-extensively drug-resistant TB (pre-XDR TB) as MDR TB with additional resistance to fluoroquinolones, and XDR TB as Pre-XDR TB with additional resistance to one additional group A drug (bedaquiline and linezolid as of July 2022) (Kherabi *et al*., 2022). In 2018, 553 cases confirmed cases of XDR-TB were notified in SA, corresponding to 5% of all multidrug-resistant/rifampicin-resistant TB (MDR/RR-TB), and about 0.18% of all TB cases (Oostvogels *et al.,* 2022). Some studies have shown that the XDR-TB epidemic in South Africa was mainly driven by the acquisition of Rif-resistant mutations (Oostvogels *et al*., 2022).

## 2.4 Diagnostic Advances in TB Drug Resistance Detection

According to Armstrong *et al.* (2023), the BD MAXTM multidrug-resistant (MDR)-TB assay (BD MAXTM) has demonstrated high sensitivity and specificity for the detection of the *Mycobacterium tuberculosis* complex (MTBC) as well as resistance to INH and Rifampin (RIF) in pulmonary specimens but has not been rigorously assessed in extrapulmonary samples. For the diagnosis of active TB in diverse low- and middle-income settings, the BD MAX MDR-TB test had a sensitivity of 93% for confirmed pulmonary TB cases, with an accuracy that appeared to be comparable to Xpert MTB/RIF (Shah *et al.,* 2019). Among the potential benefits of the BD MAX assay is the detection of mutations in the *inhA* promoter*, katG*, in addition to *rpoB*, in contrast to other commonly used molecular assays that focus on the initial identification of RIF resistance alone (Shah *et al.,* 2019). While the

distribution of INH mutations has been less well mapped globally, the WHO estimates suggest that nearly 8% of patients with TB worldwide have RIF-susceptible, INH-resistant TB. Some parts of the world may have rates of mono-resistance of more than 10–20%, with poorer treatment outcomes when treated with standard first-line regimens (Shah *et al.,* 2019).

According to Ramosubban *et al*. (2024), the mfloDx™ diagnostic platform developed by EMPE Diagnostics AB, Sweden is based on two well-established technologies: padlock probe (PLP)-dependent rolling circle amplification (RCA), an isothermal nucleic acid amplification method, and sensitive lateral-flow nucleic acid biosensor chemistry (signal development readout). mfloDx™ MDR-TB (EMPE Diagnostics AB, Stockholm, Sweden and EMPE Diagnostics Private Limited, Hyderabad, India) is a molecular test developed to detect the presence of MTB (by capturing the conserved internal transcribed spacer genomic deoxyribonucleic acid [DNA] region) and clinically significant hotspot mutations in *rpoB*, *katG*, and *inhA* coding for resistance to major first-line antibiotics RIF and INH. The mfloDx™ MDR-TB test showed a sensitivity of 86.4%, 84.9%, 65.5% and specificity of 86.1%, 92.7%, 100% in comparison with smear microscopy, MGIT culture and Xpert MTB/RIF, respectively, from both smear-positive and smear-negative samples (Ramosubban *et al.,* 2024). In smear-positive sputum samples, the mfloDx™ MDR-TB test showed a sensitivity of 92.5% and 86.4% against the MGIT culture and Xpert MTB/RIF, respectively (Ramosubban *et al.*, 2024). In addition, the mfloDx™ MDR-TB showed a 100% sensitivity and specificity for RIF susceptibility testing results when compared to MGIT DST (Ramosubban *et al.,* 2024). In comparison with Xpert MTB/RIF, mfloDx™ MDR-TB showed a sensitivity of 89% for RIF susceptibility testing (Ramosubban *et al.*, 2024).

# CHAPTER 3: METHODOLOGY

## 3.1 Study location

The Tshepong Hospital TB Referral Laboratory, located in Klerksdorp, North-West Province, has been identified as the study site for data collection in this retrospective study. As the NHLS referral TB laboratory, Tshepong provides diagnostic services to approximately 80% of the population in the North-West Province. Its central role in TB culture testing ensures access to a comprehensive and centralized dataset, making it an appropriate source for analysing drug resistance patterns in the region.

## 3.2 Research design

This study was a retrospective, quantitative investigation that utilized existing data obtained from the National Health Laboratory Service (NHLS) Central Data Warehouse (CDW). A systematic analysis was conducted on quantifiable data, and appropriate statistical techniques were applied to evaluate drug sensitivity profiling

## 3.3 Population and sampling

Patient details and laboratory episode numbers were not included in the dataset, except for age. The data included information on the instruments used to perform the tests, results for both first- and second-line drugs, and the referring facilities. Approval to access the data was obtained from the NHLS through the AARMS application, and ethical clearance was granted by the HSREC prior to submission to the NHLS for final approval. All NHLS laboratory data are stored in the Laboratory Information System (LIS) and are centrally managed through the CDW for data extraction. A stratified randomized dataset was selected for this study.

## 3.4 Study layout

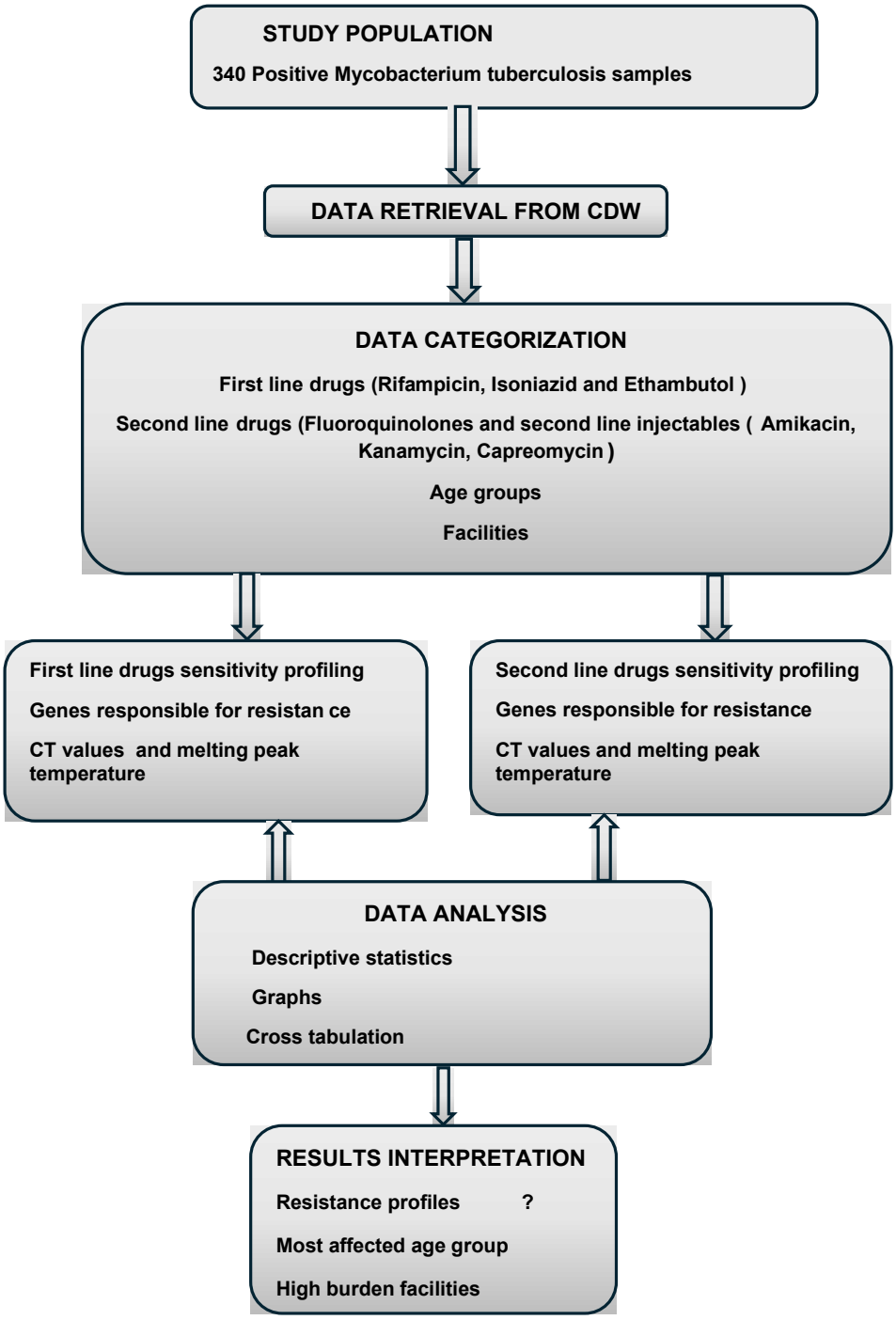


*Figure 1: Study layout for sensitivity profiling of genes associated with multi- and extensively drug-resistant Mycobacterium tuberculosis isolated from Tshepong NHLS Referral TB Laboratory, North West Province.*

## 3.5 Inclusion and exclusion criteria

### *3.5.1 Inclusion Criteria*

(i) Positive culture samples that are *Mycobacterium tuberculosis* positive.

(ii) Facilities

(iii) Patients age 18 years and older**.**

### *3.5.2 Exclusion Criteria*

(i) Negative culture samples

(ii) MOTTs samples

(iii) Biological sex (because it was not included in the data set)

## 3.6 Lab analysis methodology

The sputum samples were processed according to National Health Laboratory Service TB Laboratory Standard Operating Procedure (SOP) and according to Good Laboratory Practice (GLP). Personal Protective Equipment were used during sample processing. The samples were processed inside the Biosafety Cabinet level 2 by qualified and competent staff members. SOPs used to process the samples were as follows: MIC0462 version 10, which explains Processing of specimens for primary isolation by culture of Mycobacteria using N-Acetyl-L-Cysteine-Sodium Hydroxide (NALC-NaOH) digestion and decontamination; MIC1425 version 6, which explains BD MGIT TBc Identification test processing; GPL3695 for GeneXpert processing Operation, Maintenance and Troubleshooting; GPL4487 version 2 for GeneXpert/XDR sputum Specimen processing; and GPL4510 version 1 for BD Max Operation and MDR-TB specimen processing.

Sputum samples were decontaminated using the NALC-NaOH method. The decontamination solution consisted of 200 ml sodium hydroxide, sodium citrate, and 2 g N-acetyl-L-cysteine (NALC) powder. After decontamination, the samples were centrifuged for 20 minutes and then inoculated into the Mycobacteria Growth Indicator Tube (MGIT) liquid culture medium. Each tube was supplemented with 800 µl of PANTA (a mixture of

polymyxin B, amphotericin B, nalidixic acid, trimethoprim, and azlocillin). Smears were also prepared using the same processed samples.

The inoculated tubes were loaded into the BACTEC MGIT system for incubation and monitoring. Prepared smears were dried on a slide dryer for 30 minutes, stained using Auramine O, and examined under a fluorescent microscope to detect the presence of acid-fast bacilli (AFB).

Smears that tested positive for AFB were flagged, and their corresponding direct culture samples were separated from the AFB-negative ones. These AFB-positive samples were further processed using the Xpert® MTB/RIF Ultra assay to test for rifampicin resistance.

Samples found to be resistant to rifampicin were further analysed using the GeneXpert® MTB/XDR system to assess sensitivity to isoniazid, ethionamide, fluoroquinolones, and second-line injectable drugs such as amikacin, kanamycin, and capreomycin.

Samples that turned positive in the BACTEC MGIT system after several days of incubation were removed from the instrument, and smears were prepared. These smears were stained with carbol fuchsin and examined under an LED microscope to differentiate between *Mycobacterium tuberculosis* complex (MTBC), which forms characteristic cord-like structures, and non-tuberculous mycobacteria (NTM), formerly referred to as MOTT (Mycobacteria Other Than Tuberculosis).

All smear-positive samples were subsequently subjected to TB antigen detection assays to confirm the presence of *Mycobacterium tuberculosis* complex. Samples that tested negative for TB underwent Line Probe Assay (LPA-CM) to detect NTM species.

Samples confirmed as TB-positive were re-tested for rifampicin resistance using the GeneXpert® MTB/RIF assay. Those confirmed as rifampicin-resistant followed the same workflow as the directly detected rifampicin-resistant samples.

For further processing, BD MAX™ MDR-TB Sample Tubes were labelled with the same identification number as the original sputum containers. The BD MAX™ Sample

Treatment Reagent (SRT) was added to each sputum specimen at a 2:1 ratio (i.e. two parts reagents to one part sputum). The tubes were capped and shaken vigorously 10 times, then incubated at room temperature for 5 minutes. After incubation, the specimens were shaken again 10 times and incubated for an additional 25 minutes.

Using a sterile pipette, 2.5 ml of the treated sample was transferred into the labelled BD MAX™ MDR-TB Sample Tube. These tubes were then loaded into the BD MAX™ Instrument for automated molecular testing. The results were transmitted from instrument to LIS. Data of results from these analysis methods were then captured on the LIS and stored in CDW.

**Summary of Instruments that were used to isolate genes and the genes that will be isolated**

**Xpert MTB/XDR assay**

- ✓ *KatG* and *fabG1* genes and *InhA* promoter for Isoniazid
- ✓ *rrs* and *eis* genes second line injectable
- ✓ *GryA* and *gryB* for Fluoroguinolones
- ✓ *inhA* promoter for Ethionamide

**Xpert MTB/RIF Ultra**

- ✓ *rpoB* gene for Rifampicin

**BD MAX™ MDR-TB Assay**

- ✓ *rpoB* gene for Rifampicin
- ✓ *KatG* genes and the *inhA promoter* region

## 3.7 Data management plan

### *3.7.1 Data collection*

The data for this research included data from May 2023 to May 2025. After approval to use, NHLS data were received from the NHLS Academic Affairs and Research Management system (AARMS) and HSREC data were obtained from the Central Data Warehouse (CDW). The requested data were for patients with tuberculosis, an infection

caused by a *Mycobacterium tuberculosis* organism between May 2023 and May 2025. A total of 340 Mycobacterium tuberculosis-positive isolates were analysed in this study. Documented NHLS laboratory results recorded drug sensitivity for first-line drugs, and second-line drugs were received. The NHLS AARMS department is based in Gauteng. Since the NHLS is the data owner, no approval from North-West Department of Health was needed from the North-West University, because approval was obtained from the NHLS AARMS on behalf of NHLS managers and business managers. The research was conducted by a student of the CUT, not North-West University.

### *3.7.2 Documentation and metadata*

The Research Electronic Data Capture (REDCap) website was used to capture and analyse the data, as it is a secure web application and meets all the security policies. Thus, it is password protected, which is only known to the private investigator. Data were requested in CSV file format to ensure integrity and stored in a double password-protected file/computer on Figshare storage system as original. From RedCap, the analysis was done in Excel files that were password protected on the completely deidentified data and stored with a reference to a folder different from that with the deidentified and pseudo-anonymous information. The number was stored in a different file that does not have the metadata of the original study data and identifier. The anonymous data were analysed in Excel to create documents with my initials as principal investigator, the date created and protected with passwords.

### *3.7.3 Data analysis*

Data were presented in the form of graphs, charts and tables and patient identities were excluded due to confidentially purposes. The statistical methods were used to determine the significance of the results-based sensitivity profiling of genes responsible for multi-/extensively drug-resistant mutations in *Mycobacterium tuberculosis*. Analysis used different graphs and tables illustrating the different sensitivity profiles for the different antibiotics of the *Mycobacterium tuberculosis* organism, age affected and facility that is mostly affected. Chi-square or Fisher's exact test was used to compare resistance between RIF and INH. One-way ANOVA was used to compare melting temperatures across genes.

### *3.7.4 Data storage*

This research was low risk, but certain measures were in a place to protect original personal information in accordance with POPIA. Data were be anonymized or deidentified before being shared among the research team. Data were stored on Figshare, which is POPIA approved, to store all research securely on the cloud and enable sharing of data privately among the research team. The device was extensively scanned for malware using reputable antivirus software that could interfere with the Fishare system and regular backups could be done to ensure safe storage of data.

Before uploading of the data on Figshare, they were loaded into REDCap, which was programmed so that only the necessary deidentified data could be recorded. REDCap is encrypted and training was done for programming. Data were stored in the CUT LIS. The LIS created a subdirectory for each research study with only access to the researcher and his/her supervisor. (See attached documents for data Management plan.)

### *3.7.5 Ethics clearance*

Permission to use NHLS data was obtained from AARMS following approval of the application (NHLS AARMS reference number PR2455151). Ethical approval for the study was granted by the Health Sciences Research Ethics Committee (HSREC).Patient information and identities were not used. Data would be kept confidential with security password and access limited.

According to the Protection of Personal Information Act (POPIA) for researchers, any research involving human participants requires ethical approval from a recognised or constituted research ethics committee and that information is to be protected in accordance with sections 14, 15 and 17 of the National Health Act 61 of 2003 (RSA, 2003) with regard to confidentiality, access to health records and protection of health records.

Data supplied by the NHLS were used ethically and solely for the purpose of this research and confidentiality measures were maintained at a participant and institutional level, with no disclosure of personal or confidential information as described by the NHLS policy and POPIA.

The data were deidentified to avoid traceability to any patient. Only the information required for the research was supplied. The data used in this study were obtained from routine clinical-care investigations and no further investigations were required.

### *3.7.6 Data sharing and accountability*

The data would not be shared to anyone else outside my research group. The Private Investigator is responsible for obtaining, storing and protecting the research data according to the POPIA code of conduct. The Private Investigator will be liable for any non-compliance.

The data will be stored for 5 years and thereafter destroyed. If the Private Investigator leaves the NHLS/CUT, the data under my protection will be deleted, files deleted, and final control will be by the supervisor/CUT or employee appointed in the event that the supervisor is no longer available.

# CHAPTER 4: RESULTS

## 4.1 Baseline characteristics of the study isolates according to the Age Group and TB Classification)

The baseline characteristics of the isolates, stratified by age group and TB classification, are summarized in Table 1 and illustrated in Figures 1 and 2.

*Table 1: Baseline characteristics of the isolates according to the age group and TB Classification*

| Characteristic | Frequency (n) | Percentage (%) |
|---|---|---|
| Age Group | | |
| 18-20 | 13 | 3.8 |
| 21-30 | 70 | 20.6 |
| 31-40 | 102 | 30 |
| 41-50 | 87 | 25.6 |
| 51-60 | 51 | 15 |
| >61 | 16 | 4.7 |
| TB Classification | | |
| MDRTB | 211 | 62.1 |
| UNC | 50 | 14.7 |
| Hr-TB | 42 | 12.4 |
| DSTB | 22 | 6.5 |
| XDRTB | 8 | 2.4 |
| Poly-RTB | 7 | 2.1 |

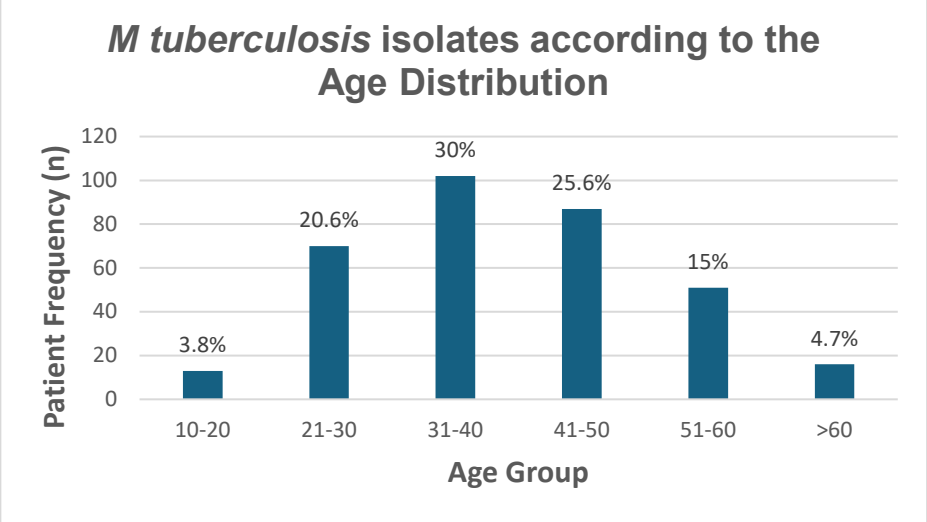


*Figure 2: Indication of M tuberculosis isolates according to the Age Distribution*

Figure 2 represents the largest proportion of cases occurred in the 31–40 years age group (30%), followed by 41–50 years (25.6%) and 21–30 years (20.6%), collectively accounting for more than 75% of all cases.

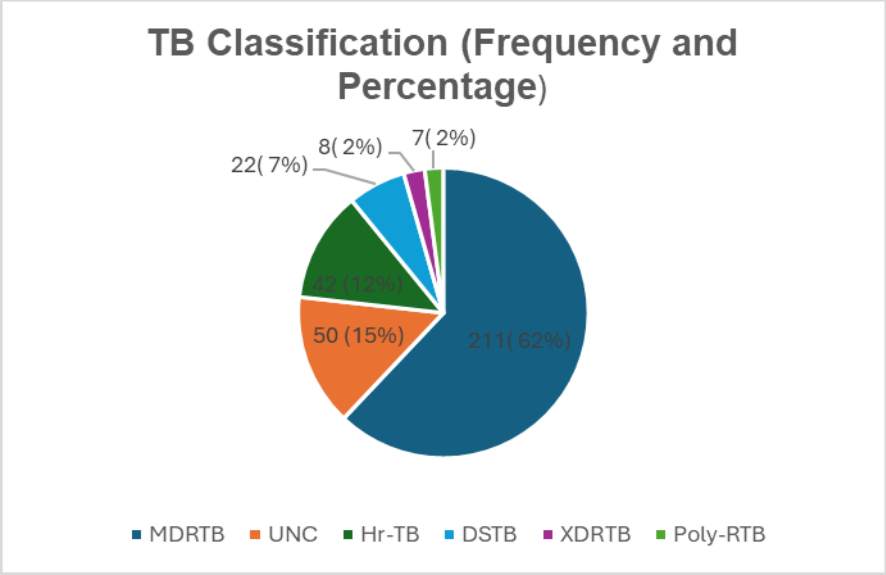


*Figure 3: M tuberculosis isolates according to the TB Classification*

Figure 3 shows that MDR-TB was the most common classification (62%), followed by unclassified cases (15%), Hr-TB (12%), DS-TB (7%), XDR-TB (2%), and poly-R-TB (2%).

## 4.2 Drug susceptibility patterns for the first line and second-line drugs

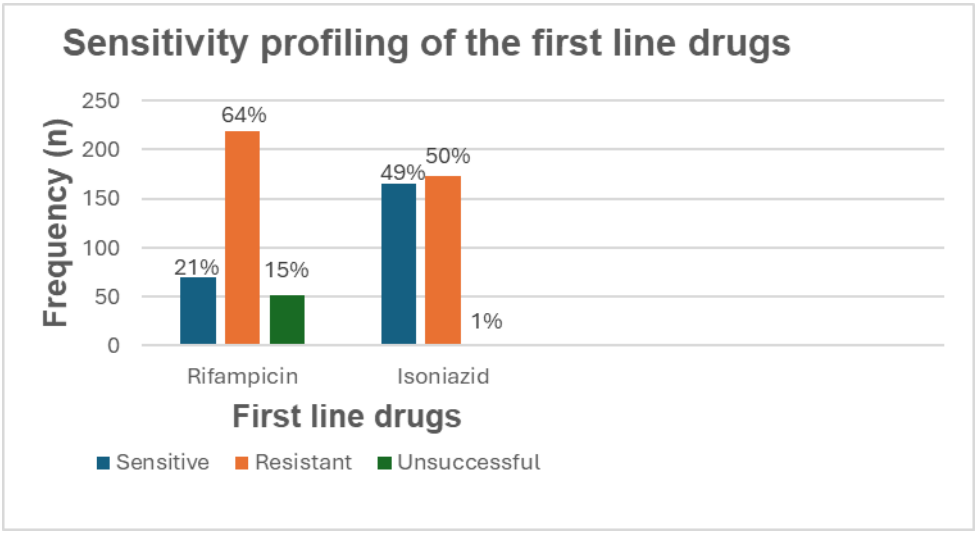


*Figure 4: Sensitive and resistant outcomes for the Rifampicin and the Isoniazid.*

Figure 4 shows that out of the 340 specimens tested, 64% (n = 219) were resistant to rifampicin, while only 21% (n = 70) were sensitive. A significant proportion (15%; n = 52) produced. Fifty per cent (50%) (n = 173) of isolates were resistant to isoniazid with 49% (n = 165) sensitive and 1% (n = 2) unsuccessful. The resistance rate for RIF was significantly higher than for INH (64% vs 50%).

*Table 2: Comparison of Resistance Between Rifampicin and Isoniazid*

| Drug | Resistant (n) | Non-Resistant (n) |
|---|---|---|
| **Rifampicin** | **219** | **121** |
| **Isoniazid** | **173** | **167** |

Table 2 indicates the comparison of resistance between rifampicin and isoniazid, which shows that frequency (n) = 219 of rifampicin which are resistant and n = 173 of isoniazid which are resistant. The resistance rates for rifampicin (RIF) and isoniazid (INH) were compared using a 2×2 contingency table. The combined number of sensitive and unsuccessful results were considered non-resistant. Chi-square test and Fisher's Exact Test were used to compare the resistance between the Rifampicin and Isoniazid. Chi-square test yielded $\chi^2 = 12.20$, $p = 0.00048$, indicating a statistically significant difference in resistance rates between RIF and INH. Fisher's Exact Test confirmed this result with $p = 0.00047$ and an odds ratio of 1.75, indicating that specimens were 1.75 times more likely to be resistant to RIF compared to INH.

*Table 3: Indication of the sensitivity profiling of the second line drugs.*

| Drugs | Sensitive (n) | Sensitive (%) | Resistant (n) | Resistant (%) | Unsuccessful (n) | Unsuccessful (%) | Total number |
|---|---|---|---|---|---|---|---|
| **Fluoroquinolones (FLQ)** | **317** | **93.2** | **21** | **6.2** | **2** | **0.6** | **340** |
| **Amikacin (AMI)** | **336** | **98.8** | **3** | **0.9** | **1** | **0.3** | **340** |
| **Kanamycin (KAN)** | **336** | **98.8** | **3** | **0.9** | **1** | **0.3** | **340** |
| **Capreomycin (CAP)** | **336** | **98.8** | **3** | **0.9** | **1** | **0.3** | **340** |
| **Ethionamide (ETH)** | **297** | **87.4** | **43** | **12.7** | **0** | **0** | **340** |

Table 3 summarizes the prevalence of resistance for second-line anti-tuberculosis drugs based on a total of 340 tested isolates. The analysis of second-line anti-tuberculosis drug susceptibility patterns indicates that ethionamide (ETH) shows the highest resistance prevalence at 12.7%, followed by fluoroquinolones (FLQ) at 6.2%. Amikacin, kanamycin, and capreomycin all share a very low prevalence of resistance (0.9%).

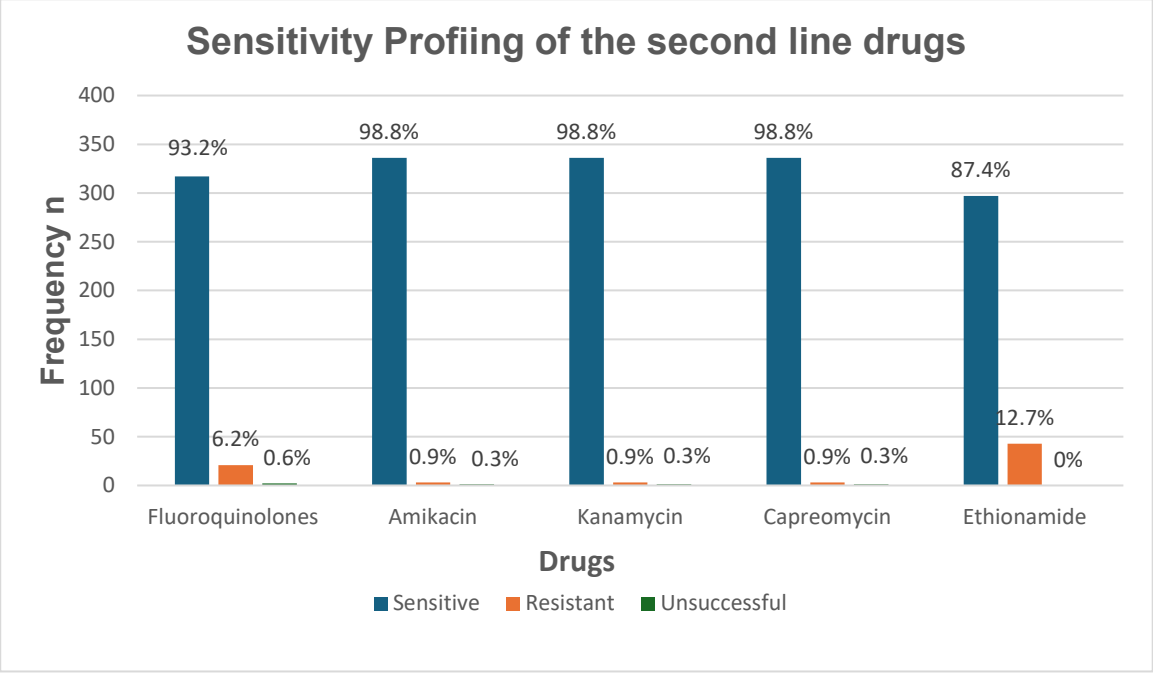


*Figure 5: Levels of sensitivity and resistant across second-line drugs*

Figure 5 demonstrate varying levels of sensitivity and resistance across second-line drugs. A majority of isolates were classified as sensitive, though resistance was also observed, and unsuccessful. The highest sensitivity levels were seen in amikacin (98.8%), capreomycin (98.8%), kanamycin (98.8%) and fluoroquinolones (93.2%). The highest resistance levels were noted in ethionamide (12.7%), compared to other drugs, i.e. fluoroquinolones (6.2%) and amikacin, capreomycin, kanamycin, which all had 0.9%.

### 4.3 Genetic Mutations Associated with Drug Resistance in *Mycobacterium tuberculosis* Based on Melting-Peak Temperature Analysis

*Table 4: Melting-Peak temperature of gene*

| Genes | Drug affected | Resistant isolates(n) | Resistant isolates (%) | Mean Melting-Peak Temperature(°C) | STD Melting-Peak Temperature(°C) |
|---|---|---|---|---|---|
| ***rpoB*** | **Rifampicin** | **219** | **64.4** | **0** | **0** |
| ***KatG*** | **Isoniazid** | **173** | **50.9** | **70** | **2.50** |
| ***inhA*** | **Isoniazid** | **173** | **50.9** | **75** | **2.20** |
| ***gyrA*** | **Fluoroquinolones** | **21** | **6.2** | **72** | **3.10** |

| | | | | | |
|---|---|---|---|---|---|
| ***Rrs*** | **Amikacin, Kanamycin, Capreomycin** | **3** | **0.9** | **71** | **0.06** |
| ***Eis promoter*** | **Kanamycin** | **3** | **0.9** | **68** | **0.06** |

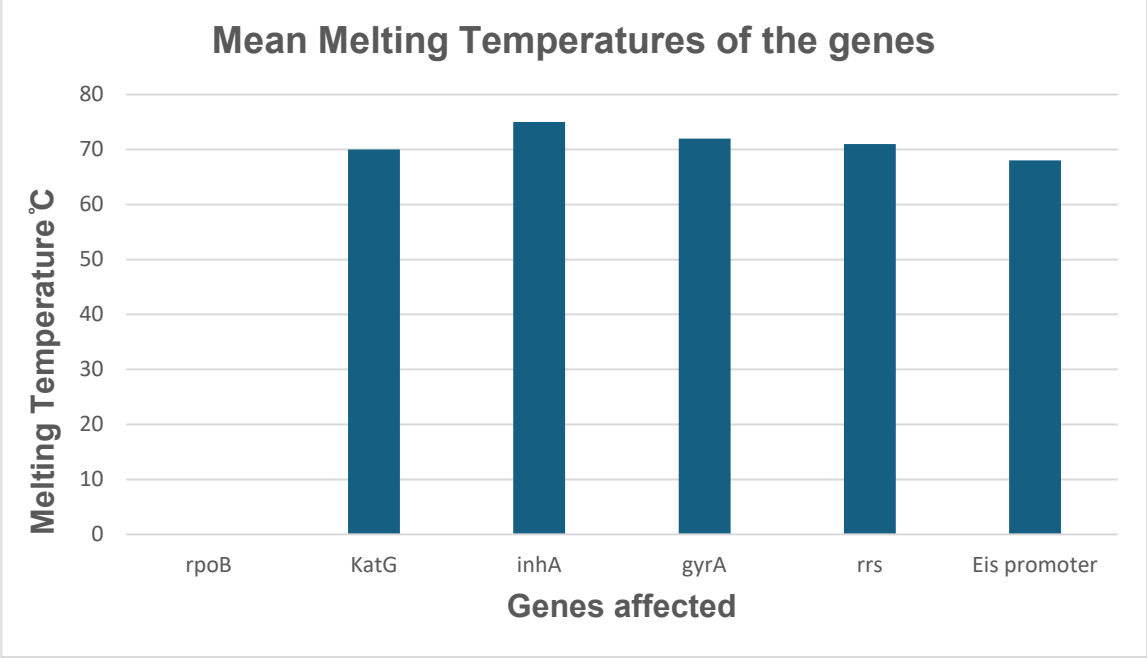


*Figure 6: Mean Melting-Peak Temperatures of Drug-Resistant-Associated Genes*

Table 4 and Figure 6 show that the *rpoB* gene for Rifampicin resistance was the gene with the highest resistant isolates (n = 219; 64.4%). Mean and standard deviation (STD) melting temperature for *rpoB* was not reported. The *KatG* gene for Isoniazid resistance was the second gene after *rpoB* with the highest resistant isolates (n = 173; 50%). The *InhA* promoter gene for Isoniazid resistance indicated the highest mean melting temperature of 75 °C, followed by the gene for fluroquinolones resistant with a mean melting temperature of 72 °C and *rrs* gene for amikacin, capreomycin and kanamycin resistance with a mean melting temperature of 71 °C. The lowest mean melting temperature of 68 °C was seen in the *Eis promoter* gene for Kanamycin resistance.

An ANOVA test was used to compare melting temperatures across the genes. One-Way ANOVA test results were as follows,

F-Statistic: 13.29

p-Value: <0.001

meaning there is a statistically significant difference in mean temperatures between the genes. It shows that the thermal stability of the mutations differs according to the gene affected. One gene has a mean that is significantly different from the others. This suggests that drug-resistance genes have different melting characteristics.

### 4.4 Distribution and Prevalence of *Mycobacterium tuberculosis* Cases by Facility and Prevalence of the MDR-TB, and XDR-TB in the North West Province isolated from the Tshepong TB referral laboratory.

*Table 5: Distribution and prevalence of MDR-TB and XDR-TB by facility.*

| Facilities (Catchment Areas) | DSTB Cases | DSTB (%) | MDR TB Cases | MDR TB (%) | XDR TB Cases | XDR (%) | Total TB Cases | Prevalence (%) TB Cases |
|---|---|---|---|---|---|---|---|---|
| Tshepong Hospital (Catchment Areas) | 10 | 6.5 | 95 | 61.3 | 5 | 3.2 | 155 | 45.6 |
| Other / Small Facilities | 6 | 10.7 | 29 | 51.8 | 1 | 1.8 | 56 | 16.5 |
| Moses Kotane Hospital Catchment Areas | 2 | 4.4 | 32 | 71.1 | 3 | 6.7 | 45 | 13.2 |
| Rustenburg Hospital Catchment Areas | 0 | 0.0 | 12 | 75.0 | 0 | 0.0 | 16 | 4.7 |
| Mafikeng Hospital Catchment Area | 0 | 0.0 | 8 | 50.0 | 0 | 0.0 | 16 | 4.7 |
| Gelukspan Catchment (Community Hospital) | 1 | 6.7 | 10 | 66.7 | 0 | 0.0 | 15 | 4.4 |
| Nic Bodenstein Catchment Areas | 1 | 8.3 | 6 | 50.0 | 1 | 8.3 | 12 | 3.5 |
| Schweizer Reneke Hospital Catchment Areas | 0 | 0.0 | 3 | 33.3 | 0 | 0.0 | 9 | 2.6 |
| Swartruggens Hospital Catchment Areas | 0 | 0.0 | 5 | 83.3 | 0 | 0.0 | 6 | 1.8 |
| Lehurutshe Catchment Areas | 0 | 0.0 | 3 | 75.0 | 0 | 0.0 | 4 | 1.2 |
| Bloemhof/Christiana Catchment Area | 0 | 0.0 | 1 | 33.3 | 0 | 0.0 | 3 | 0.9 |
| Lichtenburg Catchment Area | 0 | 0.0 | 2 | 100.0 | 0 | 0.0 | 2 | 0.6 |
| Joe Morolong Memorial Hospital | 0 | 0.0 | 1 | 100.0 | 0 | 0.0 | 1 | 0.3 |
| Grand Total | 20 | 5.9 | 207 | 60.9 | 10 | 2.9 | 340 | 100 |

Table 5 indicates the distribution and prevalence of MDR-TB and XDR-TB based on the facilities or Catchment areas. The study shows that the Tshepong Catchment area reported 155 cases (45.6%), MDR-TB accounted for 61% (n = 95), while XDR-TB was 3.2% (n = 5). The Moses Kotane Catchment areas were second with the highest cases (n = cases; 13.2%) with an MDR prevalence of 71.1% (n = 32) and XDR (n = 3; 6.7%). Other small facilities combined showed n = 56 cases with a prevalence of 16.5%. The Rustenburg and Mafikeng Catchment areas contributed 4.7% each (n =16); MDR cases for the Rustenburg Catchment area were 75% (n = 12) and 8 for the Mafikeng Catchment area with a prevalence of 50%. No cases of XDR were observed for both facilities. The Gelukspan Catchment area contributed 4.4% (n = 15) of cases with a high MDR prevalence of 66.7%. The Nic Bodenstein Catchment contributed 3.5% (n = 12) and was the only smaller catchment to report XDR cases (8.3%). The Swartruggens, Lehurutshe, Schweizer Reneke, Bloemhof/Christiana, and Lichtenburg Catchment areas had small numbers of cases (<3% each), but several had an MDR prevalence ≥ 75%, with no cases of XDR. The Joe Morolong Memorial Hospital reported only one case (0.3% prevalence) which was the smallest of all, but it was 100% MDR-TB, with no cases of XDR TB.

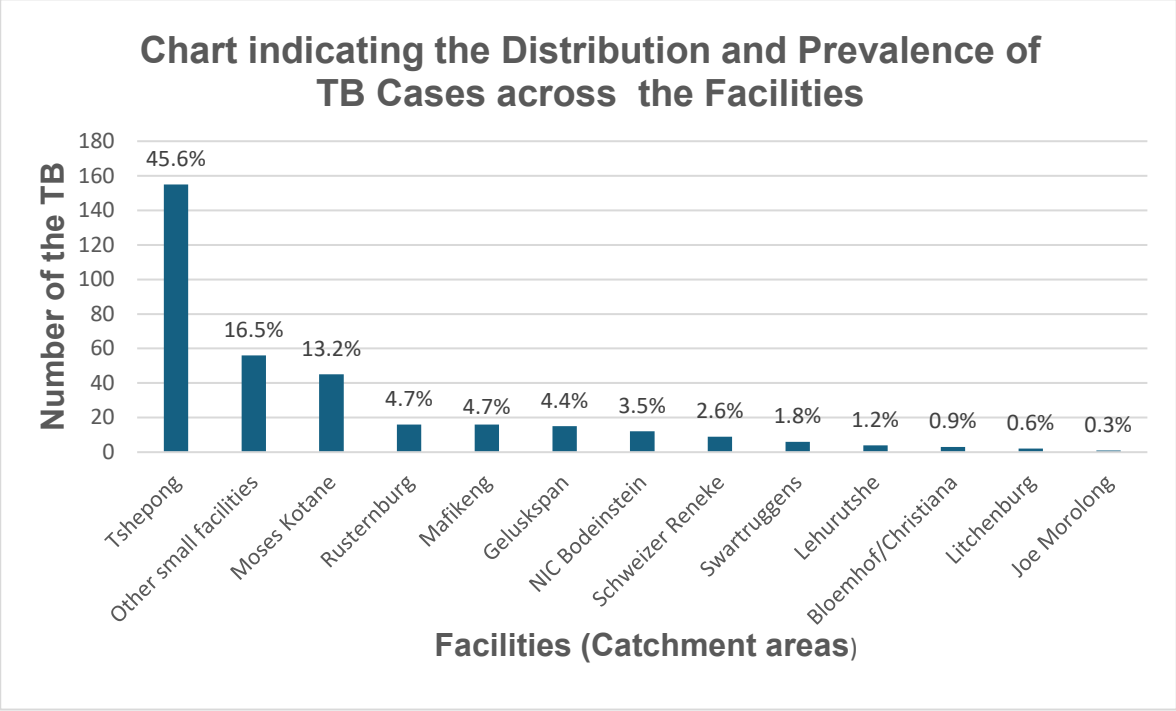


*Figure 7: Distribution and Prevalence of Total TB Cases per Catchment Area*

Figure 7 indicates the distribution and the Prevalence of Total TB Cases across the Facilities. According to the Figure, the Tshepong Catchment area accounted for n = 155 with a prevalence of 45.6%, which is the highest compared to the other facilities. The Moses Kotane Catchment area was the second-highest accounted for (n = 45), with a prevalence of 13.2%. Other small facilities combined represented n = 56 (16.5%). The Rustenburg and Mafikeng Catchment areas represented n = 16 (4.7%). The total number of cases (n = 12) with a prevalence of 3.5% was noted in the Gelukspan Catchment area, which is the smallest of all. Swartruggens (n = 6; 1.8%), Lehurutshe (n = 4; 1.2%), Bloemhof/Christiana (n = 3; 0.9%) contributed small numbers of TB cases. The Joe Morolong Memorial Hospital reported only one case (0.3%).

# CHAPTER 5: DISCUSSION AND CONCLUSION

Table 1 showed that the largest proportion of cases occurred in the 31–40 years age group (30%), followed by 41–50 years (25.6%) and 21–30 years (20.6%), which contributed for more than 75% of all cases. Figure 3 indicates that MDR-TB was the most frequent classification (62.1%), indicating a high burden of multidrug resistance in the study population. The predominance of MDR-TB indicates an ongoing public-health challenge, suggesting either an ongoing transmission of drug-resistant strains or inadequate treatment adherence, leading to resistance development. The presence of Hr-TB (12.4%) further highlights the importance of diagnostic tools that detect isoniazid monoresistance. Early identification is critical, as failure to modify treatment regimens could lead to amplification of resistance, resulting in MDR-TB. Although XDR-TB represented a smaller proportion (2.4%), it remains clinically significant, given its association with limited treatment options and poorer patient outcomes. The proportion of unclassified cases (14.7%) indicates a need for improved data capture and classification to ensure accurate reporting and targeted management.

The analysis of first-line drug sensitivity in this study highlights important patterns in the resistance and sensitivity profiles of *Mycobacterium tuberculosis* isolates. Rifampicin (RIF) and Isoniazid (INH) are very important and are the cornerstones of first-line anti-tuberculosis therapy. Figure 3 shows that out of the 340 specimens tested, 64% (n = 219) were resistant to rifampicin, while only 21% (n = 70) were sensitive. A significant proportion (15.0%; n = 52) produced unsuccessful results. The high rate of RIF resistance confirms its role as a reliable marker for multidrug-resistant TB (MDR-TB) in this population. 50% (n = 173) of isolates were resistant to isoniazid with 49% (n = 165) sensitive. Second-line drug susceptibility testing revealed that fluoroquinolone resistant was 6.2%, while resistance to second-line injectables drugs such as amikacin, kanamycin and capreomycin was 0.9%. Ethionamide resistance was recorded as the highest at 12.7%, which is a concern, as ETH is commonly used in MDR-TB regimens. Resistance may be linked to cross-resistance with INH through *inhA promoter* mutations according to Figure 5. North-West FLQ resistance rate is comparable to the global average of 16.2% reported by Kherabi *et al.* (2022) when including MDR/RR-TB isolates, but lower than

figures reported in some high-burden settings, which range from 8-15% (Diriba *et al*., 2023). The low resistance rate for second-line drugs suggests that the standard second-line regimens are still effective for the majority of the patients. Because of the resistant strains in the ETH and FLQ it is important for rapid detection and early intervention.

The analysis of melting peak temperatures across resistance-associated genes provides molecular insights into the mechanisms underlying *Mycobacterium tuberculosis* drug resistance. The findings show differently that genes are linked to the specific drug classes, and the melting peak temperature of genes differs according to the drugs affected. The isoniazid resistance was associated with mutations in the *inhA* promoter and *katG* gene, rifampicin resistance was linked to mutations in *rpoB,* fluoroquinolone resistance was conferred by *gyrA* mutations, resistance to amikacin, kanamycin, and capreomycin was associated with *rrs* mutations, and kanamycin resistance was additionally linked to mutations in the *eis promoter* region. The mean melting peak temperatures observed for each gene were consistent with expected assay performance, demonstrating assay reliability for most isolates. This study analysed the melting peak temperatures of six key resistance-associated genes (*rpoB, katG, inhA, gyrA, rrs, and eis)* from 340 *Mycobacterium tuberculosis* isolates to characterize the molecular basis of drug resistance. Table 4 indicates that for the *rpoB* gene a total of 219 isolates (64.4%) were resistant to rifampicin, confirming that RIF resistance is a major driver of MDR-TB in this study. The table reported no mean melting-peak temperature or standard deviation for *rpoB.* Despite this, the high prevalence aligns with reports that over 95% of rifampicin resistance is due to *rpoB* mutations. Table 4 also revealed 173 resistant isolates (50.9%) for both *katG* and *inhA.* The mean melting-peak temperatures were 70 °C (±2.5) for katG and 75 °C (±2.2) for *inhA,* with relatively narrow standard deviations, indicating highly reproducible assay performance. *GyrA* showed that only 21 isolates (6.2%) were resistant to fluoroquinolones, with a mean melting peak of 72 °C (±3.1), showing greater variability compared to *katG* and *inhA.* Resistance to second-line injectables was the lowest with only 3 isolates (0.9%). The mean melting peaks were 71 °C (±0.06) for *rrs* and 68 °C (±0.06) for *eis,* with very narrow standard deviations, demonstrating excellent assay reproducibility and confirming the ongoing effectiveness of aminoglycosides and capreomycin in this study. The descriptive statistics revealed stable Mean Melting Peaks

for *inhA* and *katG*, suggesting reproducibility in detecting isoniazid resistance. An ANOVA test was used to compare melting temperatures across the genes.

There is a statistically significant difference in mean temperatures between the genes according to the ANOVA test, which revealed F-Statistic 13.29 and p-Value <0.001. It shows that the thermal stability of the mutations differs according to the gene affected. One gene has a mean that is significantly different from the others. This suggests that drug-resistance genes have different melting characteristics.

Tshepong was the leading contributors of MDR-TB cases, followed by Moses Kotane. Tshepong Hospital and Moses Kotane Hospital recorded the highest number of MDR-TB cases (95 and 32 cases, respectively), jointly contributing over 80% of the total MDR-TB cases in this dataset. These results mirror findings by Worku *et al.* (2022) and Bisimwa et al. (2021), who observed that MDR-TB prevalence tends to cluster in facilities with higher diagnostic capacity and patient volume. The relatively high MDR prevalence at the Lichtenburg Catchment Area and Joe Morolong (100%), despite a small number of total cases, suggests that even smaller facilities can be important foci for transmission a trend also reported by Nandlal *et al.* (2022), who note that MDR-TB transmission is increasingly driven by person-to-person spread rather than treatment-acquired resistance. The Bloemhof/Christiana Catchment reported low MDR rates (33%). The Tshepong, Moses Kotane and NIC Bodenstein Catchment areas and other small facilities were only facilities with XDR, with Tshepong (n = 5) prevalence of 3.2% and Moses Kotane (n = 3) prevalence of 6.7% being the highest.

The Tshepong Catchment accounted for the largest proportion of TB cases (n = 155; 45.6%), reflecting its role as the main referral hospital in the North-West Province and the concentration of complicated TB cases from surrounding facilities. The Moses Kotane Catchment area was the second-largest contributor (n = 45; 13.2%), highlighting a significant TB burden in Mogwase and surrounding areas. The Rustenburg and Mafikeng Catchments each contributed 4.7%, indicating moderate burdens. Smaller contributors included the Gelukspan (4.4%) and Nic Bodenstein (3.5%) Catchments, which still reported MDR and XDR cases, confirming that drug resistance is present even in low-volume settings. Swartruggens (1.8%), Lehurutshe (1.2%), Bloemhof/Christiana (0.9%),

and Lichtenburg (0.6%) had very low case counts, but still warrant continued monitoring to prevent transmission. The Joe Morolong Memorial Hospital reported only one case (0.3%), which was 100% MDR-TB, emphasizing the need for case follow-up and investigation to rule out missed cases.

## 5.1 Conclusion

The study was all about the “Sensitivity profiling of genes responsible for multi-/extensively drug-resistant mutations in *Mycobacterium tuberculosis* isolated from the Tshepong National Health Laboratory Service Referral Tuberculosis Laboratory in North-West”. The study looked for baseline characteristics of *Mycobacterium tuberculosis* isolates, sensitivity profiling of the first- and second-line drugs, genes responsible for drug resistance according to the Melting Peak temperature and the prevalence of TB cases, MDR/XDR across different facilities. The results provide comprehensive answers to the hypotheses set out in this study

**Hypothesis 1:** A significant proportion of *Mycobacterium tuberculosis*-positive specimens are resistant to at least one first-line anti-tuberculosis drug (supported ), because the study confirmed a high proportion of resistance to rifampicin (64.4%) and isoniazid (50.9%), indicating that MDR-TB remains a significant challenge.

**Hypothesis 2:** Specimens resistant to first-line drugs are more likely to demonstrate resistance to second-line drugs, contributing to multidrug-resistant (MDR) and extensively drug-resistant (XDR) TB cases (Supported), while second-line resistance was detected – notably 6.2% fluoroquinolone resistance and 12.7% ethionamide-resistance XDR-TB cases were identified in facilities like Tshepong and Moses Kotane.

**Hypothesis 3:** Drug resistance in *Mycobacterium tuberculosis* is significantly associated with specific genetic mutations detectable through molecular diagnostic data (Supported). Melting-peak temperature analysis confirmed the presence of key resistance-conferring mutations: *rpoB* for rifampicin, *katG/inhA* for isoniazid, *gyrA* for fluoroquinolones, and *rrs/eis* for injectables.

**Hypothesis 4:** The prevalence of drug-resistant TB differs significantly across age groups, with certain age categories being more affected than others (Supported). Most of the MDR-TB and drug-resistant cases occurred in adults aged 31–40 years.

**Hypothesis 5:** The burden of drug-resistant TB is unevenly distributed across regions and healthcare facilities in the North-West Province, with certain areas/facilities showing higher case counts (Supported). The analysis showed that the Tshepong and Moses Kotane Hospitals accounted for over 80% of MDR-TB cases, confirming a clustered distribution. Smaller facilities such as NIC Bodenstein also demonstrated high MDR prevalence, despite low absolute case numbers. These findings highlight priority sites for infection control and intensified case-finding efforts.

This study demonstrates that MDR-TB remains a significant public-health threat in the North-West Province, with evidence of emerging XDR-TB cases. The high prevalence of rifampicin resistance highlights the need for routine use of rapid molecular diagnostics and comprehensive drug susceptibility testing. Strengthening TB surveillance, optimizing treatment regimens, and targeting interventions at high-burden facilities and among working age adults are essential to reduce the spreading of drug-resistant TB and improving treatment outcomes. These findings provide critical evidence for policymakers and health authorities to strengthen provincial TB control strategies.

## 5.2 Study limitations

This study was limited by its retrospective design, which relied on routinely collected laboratory data that may have been subject to reporting errors or missing results, especially Rifampicin sensitivity. Biological sex was not included in the dataset, limiting gender-based analysis of TB-resistant patterns. *rpoB* gene melting peak temperature was not also included which limited analyses of the *rpoB* gene based on melting peak temperature. Additionally, some facilities had small sample sizes, which may have led to the over- or under-estimation of prevalence rates.

## 5.3 Recommendations

Based on the findings it is recommended that routine drug susceptibility testing be done to all the culture-positive TB cases to enable early detection of drug resistance. The sputum samples should be repeated for all the results that yield unsuccessful results for better, reliable diagnosis and treatment purposes. Facilities with a high MDR-TB prevalence should receive prioritized resources for rapid diagnostics, patient education, and adherence support. Strengthening contact tracing and implementing community-based screening could help to identify undiagnosed cases earlier. All the patients that they suspect have TB based on the symptoms should get tested as soon as possible to prevent any delay in the treatment. Provincial health authorities should ensure an adequate drug supply for individualized treatment regimens and monitor emerging resistance trends to second-line drugs. Further research should include treatment outcome data and genetic sequencing to understand transmission dynamics better.

# REFERENCES


1. Alsayed, S.S.R. and Gunosewoyo, H. 2023. Tuberculosis: Pathogenesis, Current Treatment Regimens and New Drug Targets. *International Journal of Molecular Sciences* 24: 5202.

2. Armstrong, D., Fishers., Totten, M. and Parrish, N. 2023. An analytic feasibility study of the BD MAX™ MDR-TB assay for testing of non-sputum specimens for detection of the *Mycobacterium tuberculosis* complex (MTBC) and isoniazid (INH) and rifampin (RIF) resistance. *Diagnostic Microbiology and Infectious Disease* 101(1): 1-5.

3. Bisimwa, B.C., Nachega, J.B., Warren, R.M., Theron, G., Metcalfe, J.Z., Shah, M., Diacon, A.H., Sam-Agudu, N.A., Yotebieng, M., Bulabula, A.N.H., Katoto, P. D.M., Chirambiza. J., Nyota, R., Birembano, F.M., Musafiri, E.M., Byadunia, S., Bahizire, E., Kaswa M.K., Callens, S. and Kashongwe, Z.M. 2021. Xpert *Mycobacterium tuberculosis*/Rifampicin–Detected Rifampicin Resistance is a Suboptimal Surrogate for Multidrug-resistant Tuberculosis in Eastern Democratic Republic of the Congo: Diagnostic and Clinical Implications. *Clinical Infectious Diseases* 73(2): e362-e370.

4. Dahl, V.N., Butova, T., Rosenthal, A.A., Grinev, A., Gabrielian, A., Vashakidze, S., Shubladze, N., Toxanbayeva, B., Chingissova, L., Crudu, V., Chesov, D., Kalmambetova, G., Saparova, G.C., Wejse, C.M. and Dmytro, B.D. 2024. Drug-Resistant Tuberculosis, Georgia, Kazakhstan, Moldova, and Ukraine, 2017-2022. *Emerging Infectious Disease* 30(4):831-833.

5. Diriba, G., Alemu, A., Yenew, B., Tola, H.H, Gamtesa, D.F., Mollalign, H., Eshetu, K., Moga, S, Abdella, S., Tollera, G., Kebede, A. and Dangisso, M.H. 2023. Epidemiology of extensively drug-resistant tuberculosis among patients with multidrug-resistant tuberculosis: A systematic review and meta-analysis. *International Journal of Infectious Disease* 132: 50-63.

6. Gill, C.M., Dolan,L., Piggott,M.L and Mclaughhlim, A.M. 2021. New development in tuberculosis diagnosis and treatment. *Breath Review* 18: 1-5.

7. Huang, Y., Ai, L., Wang, X., Sun, Z. and Wang, F. 2022. Review and Updates on the Diagnosis of Tuberculosis. *Journal of Clinical Medicine* 11: 5826.

8. Kanabalan, R.D., Lee, L.J., Lee, T.Y., Chong, P.P., Hassan, L., Ismail, R. and Chin, K.V. 2021. Human tuberculosis and Mycobacterium Complex: A review on genetic diversity, pathogenesis, and omics approaches in host biomarkers discovery. *Elsevier* 246: 3-18.

9. Kherabi, Y., Jachym, M.F., Rioux, C., Yazdanpanah, Y., Mechai, F., Pourcher, V., Robert, J. and Guglielmetti, L. 2022. Revised Definition of Tuberculosis Resistance and Treatment Outcome, France 2006–2019. *Emerging Infectious Diseases* 28(9): 1797.

10. Kunping, C., Xiaoxiao, Z., Wei, L. and Lang, B. 2025 .Global, Regional ,and national burden and trends of multidrug-resistant tuberculosis and extensively drug-resistant tuberculosis in adolescent and adults aged 15–49 years from 2010 to 2021:insights from the global burden of disease study 2021.*BMC Medicine* 23: 445.

11. Ludi, Z., Sule, A.A., Samy, R.P., Putera, I., Schrijver, B., Hutchinson, P.E., Gunaratne, J., Verma, I., Singhal, A., Nora, D.R., Martin van Hagen, P., Dik, W.A.; Gupta, V. and Agrawal, R. 2023. Diagnosis and biomarkers for ocular tuberculosis: From the presence into the future. *Theranostics* 13(1): 2089.

12. Mirzayev, F., Viney, K., Linh, N.N., Gonzalez-Angulo, L., Medea Gegia ,M., Jaramillo, E., Zignol, M. and Tereza Kasaeva, T. 2021. World Health Organization recommendations on the treatment of drug-resistant tuberculosis, 2020 update. *European respiratory Journal* 57(6): 2-19.

13. Moule, M.G. and Cirillo, J.D. Mycobatecterium tuberculosis Dissemination Plays a critical Role in Pathologenesis. *Frontiers in Cellular and Infection Microbiology* 10: 1-6.

14. Nandlal, L., Perumal, R. and Naido, K. 2022. Rapid Molecular Assay for the Diagnosis of Drug-Resistant Tuberculosis. *Infection and Drug Resistance* 15: 4971-4984.

15. Nasiri, M. J. Amiri, M., Cheraghi, M., Silva, D.R., Sotgiu, G., D'Ambrosio, L., Centis, R., Mileva-Lopez, M., Hill, T.M., Gidey, S., Diaby, K., Hittel, N., Gandhi, H. and Dara, M. 2025. 15-year trends in efficacy and effectiveness of treatment outcomes in drug-resistant pulmonary TB. *IJTLD OPEN* 2(4): 187–198.

16. Nogueira, B.M.F., Krishnan, S., Duarte, B.B., Pereira, M.A., Queiroz, A. T.L., Ellner, J.J., Salgame, P., Scriba, T., Sterling, R.T., Gupta, A. and Andrade, B.B. 2022. Diagnostic biomarkers for acute tuberculosis: progress and challenges. *EMBO Molecular Medicine* 14: 1-13.

17. Oostvogels, S., Ley, S.D., Heupirik, T.H., Dippenaar, A., Streicher, E.M., Devos, E., Mechan, C.J., Dheda, K., Warren, R. and Van Rie, A.2022.Transmission distribution and drug resistance-conferring mutations of extensively drug-resistant tuberculosis in the Western Cape Province, South Africa. *Microbal Genomic* 8: 1-11.

18. Ramosubban, G., Michael, S.J., Gupta, R., Venkaleson, M., Beauton, A.P., Hoffner, S. and Asalapuram, P. 2024. Rapid Detection of M. tuberculosis and Its Resistance to Rifampicin and Isoniazid with the mfloDx™ MDR-TB test. *International Journal of Mycobacteriology* 13(1): 91-95.

19. Rasool, G., Khan, M.A., Mohy-Ud-Din, R. and Riaz, M. 2019. Detection of *Mycobacterium tuberculosis* in AFB smear-negative sputum specimens through MTB culture and GeneXpert MTB/RIF assay. *Sage Journals* 33: 1-4.

20. Republic of South Africa [RSA]. 2003. National Health Act 61 of 2003. Pretoria: Government Printer.

21. Rossini, N.O. and Dias, M.V.B. 2023.Mutations and insights into the molecular mechanisms of resistance of *Mycobacterium tuberculosis* to first-line. *Genetics Molecular Biology* 46(1): 20220261.

22. Saderi, L., Puci, M., Lorenzo, B.D., Centis,R., Ambrosio, L.D., Akkerman, O.W., Alffenaar, J.C., Caminero, J.A., Chakaya, J.M., Denholm, J.T., Kurhasani, X., Ong, C.W.M., Rendon, A., Silva, D.R., Tiberi, S., Zenner, D., Cabibbe, A.M., Migliori, G.B. and Sotgiu, G. 2022.Rapid Diagnosis of XDR and pre-XDR TB: *Spanish Society of Pulmonology and Thoracic Surgery (SEPAR).*

23. Shah, M., Paradis, S., Betz, J., Beylis, N., Bharadwaj, R., Caceres, T., Gotuzzo, E., Joloba, M., Mave, V., Nakiyingi, L., Nicol, P.M., Pradhan, N., King, B., Armstrong, D., Knetch, D., Maus, C.E., Cooper, C.K., Susan, E., Dorman, S.E., Yukari, C. and Manabe, Y.C. 2019. Multi-center Study of the Accuracy of the BD MAX™ MDR-TB Assay for Detection of *Mycobacterium tuberculosis Complex* and Mutations Associated with Resistance to Rifampin and Isoniazid. *Clinical Infectious Diseases* 71(5): 1161-1167.

24. Silva, D.R., Rabahi, M.F., Sont'Anna, C.C., Silva-Junior, J.L.R., Capone, D.; Bombavda, S., Miranda, S.S., Rocha, J.L., Dalcolmo, M.M.P,, Rick, M.F., Santos, A.P., Dalcin, P.T.R., Galvao, T.S. and Mello, F.C.Q. 2021.Diagnosis of tuberculosis a consensus statement from the Brazilian Thoracic Association. *Journal Brazil of Pneumonia* 47(2): 2-4.

25. Singh, P., Jamal, S., Ahmed, F., Saqib, N., Mehra, S., Ali, W., Roy, D., Ehlesham, N.Z. and Hasnain, S.E. 2021. Computational modeling and bioinformatics analyses of functional mutations in drug targets genes in Mycobacterium tuberculosis. *Computational and Structural Biotechnology* 19: 2423-2446.

26. Singh, R., Dwivedi, S.P., Gaharwar, U.S., Meena, R., Rajamani, P. and Prasad, T. 2019. Recent Updates on drug resistance in *Mycobacterium tuberculosis*. *Journal of Applied Microbiology* 128: 1547-1567.

27. Srivastava, S., Chapagain, M. and Gumbo, T. 2021. Effect of specimen processing, growth supplement, and different metabolic population on *Mycobacterium tuberculosis* laboratory diagnosis. *Plos one* 10: 1371.

28. Traoré, A.N., Rikhotso, M.C., Mphaphuli, M.A., Patel, S.M., Mahamud, H.A., Kachienga, L.O., Kabue, J. and Potgieter, N. 2023. Isoniazid and Rifampicin Resistance-Conferring Mutations in *Mycobacterium tuberculosis* Isolates from South Africa. *Pathogens* 12(8): 1015.

29. Wobudeya, E., Bonnet, M., Walters, E.G., Nabeta, P., Song, R., Murithi, W., Mchembere, W., Dim, B., Taguebue, J.V., Ciliemann, J.O., Nicol, M.P. and Marcy, O. 2022. Diagnostic Advances in Childhood Tuberculosis-Improving Specimen Collection and Yield of Microbiological Diagnosis for Intrathoracic Tuberculosis. *Pathogens Journal* 11: 1-19.

30. Worku, G., Gumi, B., Girma, M., Mohammedbirhan, B., Diriba, G., Seid, G., Getu, M., Amare, M., Sinshaw, W., Ashagre, W., Tschopp, R., Carruth, L. and Ameni, G. 2022. Drug sensitivity of clinical isolates of *Mycobacterium tuberculosis* and its association with bacterial genotype in the Somali region, Eastern Ethiopia. *Frontiers in the Public Health* 10: 01-10.

# APPENDICES

## Appendix A: Publication Certificate

International Journal of Environmental Sciences,
ISSN: 2229-7359

CERTIFICATE
OF PUBLICATION

This certificate is proudly presented to
M.J Ntanjane, B Malope- Kgokong, PM Makhoahle

In recognition of the publication of the paper entitled
Literature Review On Multi-Extensively Drug-Resistant Mycobacterium Tuberculosis Mutations: Highlighting Molecular Analysis Gaps At Tshepong NHLS Referral Laboratory.

has been published in International Journal of Environmental Sciences (2229-7359)

Volume 11 Issue 18s (2025) , Date of Publication : August 2025

ELSEVIER

MIHAI V. PUTZ
Editor in Cheif

## Appendix B: Copy of Published Article

International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 19s, 2025
https://theaspd.com/index.php

# Literature Review On Multi-Extensively Drug-Resistant Mycobacterium Tuberculosis Mutations: Highlighting Molecular Analysis Gaps At Tshepong NHLS Referral Laboratory.

**M.J Ntanjane[1,2], B Malope-Kgokong[3], PM Makhoahle[1]**
[1]Faculty of Health and Environmental Sciences, Central University of Technology-Free State, South Africa
[2]Tshepong National Health Laboratory Service Referral Tuberculosis Laboratory Klerksdorp North-West, Province, South Africa
[3]National Health Laboratory Service, Johannesburg, South Africa

***Abstract***
*The emergence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) Mycobacterium tuberculosis presents a significant challenge to global public health. This study explores the sensitivity profiling of genes responsible for MDR and XDR mutations in M. tuberculosis isolates from the Tshepong National Health Laboratory Service Referral Tuberculosis Laboratory in North West. A literature review was conducted using databases such as Google Scholar, PubMed, and PubMed Central to identify relevant articles published between 2020 and 2024. The selected studies were analysed to determine the genes involved in resistance mechanisms and to develop a conceptual framework for understanding their interactions with first- and second-line anti-tuberculosis drugs.*
*According to the literature, more than 95% of rifampicin resistance is associated with rpoB gene alterations within an 81 bp region. Additionally, isoniazid (INH) resistance is more complex and has been linked to multiple genes, particularly katG and the inhA promoter region. The reviewed studies highlight gaps in research and suggest directions for future studies to improve drug susceptibility testing, particularly in regions such as North West Province, South Africa.*
***Keywords***: *Mycobacterium tuberculosis, Multidrug-resistant, extensively drug-resistant, resistance mechanism , multiple gene*

## INTRODUCTION

Mycobacteriaceae family comprises a diverse group of bacteria with varying degrees of pathogenicity in humans and animals. These bacteria exhibit distinct host reservoirs and growth dynamics in culture (Karabala et.al., 2021). The Mycobacterium tuberculosis complex (MTBC) is a genetically related group of mycobacteria that includes Mycobacterium tuberculosis (M. tuberculosis), Mycobacterium africanum (M. africanum), Mycobacterium bovis (M. bovis), Mycobacterium canettii (M. canettii), Mycobacterium microti (M. microti), Mycobacterium pinnipedii (M. pinnipedii), and Mycobacterium caprae (M. caprae) (Karabala et.al., 2021).
Tuberculosis (TB), caused by M. tuberculosis, is a major global public health concern. It is a chronic infectious disease primarily transmitted through airborne droplets from coughing or sneezing (Rasool, 2019). TB primarily affects the lungs (pulmonary TB) but can also impact other organs (extrapulmonary TB) (Rasool, 2019). Active TB commonly presents as a persistent cough lasting several weeks, often with blood-streaked sputum. Other classic symptoms include fever, chills, night sweats, weakness, and unintentional weight loss (Moule & Cirillo, 2020). In contrast, latent TB remains asymptomatic, and individuals may be unaware of their infection unless reactivation occurs (Moule & Cirillo, 2020).
Enhancing TB diagnostic efficiency is crucial for improving treatment outcomes (Gill et.al., 2021). Various diagnostic techniques—including microscopy, culture, and molecular methods—are used to detect Mycobacterium in respiratory specimens, each with advantages and limitations (Srivastava et.al., 2020). Culture-based detection remains the gold standard due to its high sensitivity, but it is susceptible to contamination, requiring decontamination steps that can compromise bacterial viability (Srivastava et.al., 2020). The NALC-NaOH decontamination method is widely used, and a cocktail of antibiotics (PANTA: polymyxin B, amphotericin B, nalidixic acid, trimethoprim, and azlocillin) is employed to suppress non-mycobacterial contaminants (Srivastava et al., 2020). Liquid-based culture systems significantly reduce detection time, averaging 12.8 days compared to 25.1–25.5 days for solid media (Gill et.al., 2021). GeneXpert MTB/RIF is a rapid, automated polymerase chain reaction (PCR) test that detects active TB and rifampicin resistance within approximately two hours, requiring minimal training and biosafety measures (Nogueira et.al., 2022). Smear microscopy, one of the oldest TB detection methods, remains widely used due to its simplicity, speed, and cost-effectiveness. It is a cornerstone of the WHO DOTS (Directly Observed Treatment, Short-Course) strategy, utilizing Ziehl-Neelsen or fluorescence microscopy with light-emitting diodes (Wobudeya et.al.,2022).

# Appendix C: NHLS approval letter ref PR24551513

Academic Affairs and Research
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Web: www.nhls.ac.za

**18 May 2025**

**Applicant:** Motshidisi Ntanjane
**Institution:** NHLS
**E-mail Address:** ntanjane@hotmail.com
**Cell:** 083 856 8131

**Project Title:** Sensitivity profiling of genes responsible for multi-/extensively drug resistant mutations in Mycobacterium tuberculosis isolated from the Tshepong National Health Laboratory Service Referral Tuberculosis Laboratory In North West.

**Reference Number:** PR2455141

**Research Application Type(s):**
1. Request for Data

**RE: APPROVAL LETTER: REQUEST TO ACCESS NHLS RESOURCES FOR RESEARCH PURPOSES**

This letter serves to advise that the application requesting permission to conduct the above-mentioned research using the listed NHLS resources has been reviewed and **"Approved"**. Please note that the approval is granted on the condition that you comply with the NHLS Research Material and Data Access Policy and requirements stated below.

1. All material and data requested shall be used as per the research protocol submitted to the NHLS and as approved by the relevant Health Research Ethics Committee (HREC) in South Africa.
2. Access to the NHLS material and/or data shall be limited to the minimum required for successful completion of the approved study and shall be made available ***without patient names and other patient identifiers (including, but not limited to, national identity numbers, hospital/clinic file numbers, addresses and telephone numbers)***.
3. Confidentiality shall be maintained at the participant and institutional level and there shall be no disclosure of personal information or confidential information.
4. Data and/or material shall not be shared with other parties unless approved by the NHLS
5. The material and/or data obtained from the NHLS shall be anonymised and not, for any reason, be used to track or recruit patients as no pre-approval/consent is obtained from patients.
6. Processes shall be discussed with the relevant NHLS departments (i.e. Corporate Data Warehouse (CDW), NHLS Laboratory Management, Operations Office, etc.) and agreed upon.
7. Any amendments to the study requirements, including the use of the material and/or data for purposes not initially disclosed to the NHLS) shall be cleared by an approved HREC and submitted to the NHLS for approval via the AARMS system – https://aarms.nhls.ac.za.
8. The NHLS shall be acknowledged as a source of material and/or data in any output, such as abstracts and journal articles, emanating from the project.
9. A final report of the research study and any published output resulting from this study shall be submitted to the NHLS via AARMS

Please note that this letter constitutes approval by the NHLS Academic Affairs and Research Office. The NHLS entities tasked with providing the material and/data may have additional requirements for access. Data related queries may be directed to NHLS CDW, email: zarina.sabat@nhls.ac.za; contact number: 011 386 6074.

**Dr Babatyi-Matope-Kgokong**
**National Manager: Academic Affairs and Research**

## Appendix D: HSREC ethical clearance

UNIVERSITY OF THE FREE STATE
UNIVERSITEIT VAN DIE VRYSTAAT
YUNIVESITHI YA FREISTATA

UFS HEALTH SCIENCES

**Health Sciences Research Ethics Committee**

23-Jun-2025

Dear **Ms Motshidisi Ntanjane**

Ethics Clearance: **Sensitivity profiling of genes responsible for multi-/extensively drug resistant mutations in Mycobacterium tuberculosis isolated from the Tshepong National Health Laboratory Service Referral Tuberculosis Laboratory in North West.**

Principal Investigator: **Ms Motshidisi Ntanjane**

Department: **CUT - Central University of Technology**

Submission Page

**APPLICATION APPROVED**

Please ensure that you read the whole document.

With reference to your application for ethical clearance with the Faculty of Health Sciences, I am pleased to inform you on behalf of the Health Sciences Research Ethics Committee that you have been granted ethical clearance for your project.

Your ethical clearance number, to be used in all correspondence is:**UFS-HSD2024/1195/2406**

The ethical clearance number is valid for research conducted for one year from issuance. Should you require more time to complete this research, please apply for an extension.

We request that any changes that may take place during the course of your research project be submitted to the HSREC for approval to ensure we are kept up to date with your progress and any ethical implications that may arise. This includes any serious adverse events and/or termination of the study.

A progress report should be submitted within one year of approval, and annually for long term studies. A final report should be submitted at the completion of the study.

**Research conducted in any Department of Health facility**: Researchers are required to sign and return the HSREC approval letters to the provincial Department of Health where they applied. It is also a requirement for researchers to submit electronic copies of their final research findings, and/or make a presentation of their findings and recommendations at departmental research days when and where indicated.

The HSREC functions in compliance with, but not limited to, the following documents and guidelines: The SA National Health Act. No. 61 of 2003; Ethics in Health Research: Principles, Structures and Processes (2015); SA GCP(2020); Declaration of Helsinki; The Belmont Report; The US Office of Human Research Protections 45 CFR 461 (for non-exempt research with human participants conducted or supported by the US Department of Health and Human Services- (HHS), 21 CFR 50, 21 CFR 56; CIOMS; ICH-GCP-E6 Sections 1-4; International Council for Harmonisation (ICH) Harmonised Guideline, Integrated Addendum to ICH E6(R1), Guideline for Good Clinical Practice (GCP) E6(R2), 2016, SAHPRA Guidelines as well as Laws and Regulations with regard to the Control of Medicines, Constitution of the HSREC of the Faculty of Health Sciences.

The Principal Investigator (PI) bears final responsibility for the RIMS application. In the event of any misconduct or improper activities perpetuated by a third party, the PI will be held vicariously liable. The HSREC will bear no responsibility or liability for any actions of a PI and/or third party or breach of confidentiality caused by the PI and/or third party.

For any questions or concerns, please feel free to contact HSREC Administration: 051-4012650/9860 or email EthicsFHS@ufs.ac.za.

Thank you for submitting this proposal for ethical clearance and we wish you every success with your research.

Yours Sincerely

Dr. C. Armour (Barrett)
Chairperson: Health Sciences Research Ethics Committee

## Appendix E: Proof of registration

Central University of Technology, Free State

REGISTRAR

**PROOF OF REGISTRATION**
**To Whom It May Concern**

14-Feb-2025

It is hereby confirmed that the under mentioned person is a registered student at CENTRAL UNIVERSITY OF TECHNOLOGY.

**Student Number:** 211058297
**Student ID Number:** 9012260427089
**Name:** MOTSHIDISI JEANETT NTANJANE
**Registered for Period:** 06-Jan-2025-12-Dec-2025
**Qualification:** M_HBIO MASTER OF HEALTH SCIENCES IN BIOMEDICAL

| Subject | Description | Qual. | Class Group | Exam Year | Exam Month | Cancel | Offering Type | Amount |
|---|---|---|---|---|---|---|---|---|
| THESOAT | 22 DISSERTATION/THESIS DISSERTATION | M_HBIO | A | 2025 | 10 | N | 01 | 31750.00 |
| | | | | | | | Subtotal: | 31750.00 |
| | | | | | | | Total: | 31750.00 |

Outstanding Balance:34668.00

REGISTRAR

OFFICE OF THE REGISTRAR
14-Feb-2025
Central University of Technology, Free State

Disclaimer: The information contained in this proof of registration document is intended as proof that the student indicated above is registered with the Central University of Technology, Free State. It is confidential, privileged, and only for the information of the intended recipient such as donors, bursars, etc., and may not be used, published, or redistributed without the prior written consent of the student.DESTEST

## Appendix F: Supervisor Letter

Central University of Technology, Free State

Central University of Technology, Free State

FACULTY OF HEALTH AND ENVIRONMENTAL SCIENCES

To Whom It May Concern,

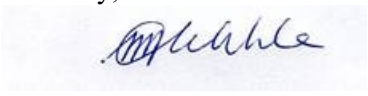
9/25/2025

This letter serves to confirm that (Motshidisi Jeanett Ntanjane, 211058297 registered for masters of health Sciences at Central University of Technology presented a protocol titled ( Sensitivity profiling of genes responsible for multi-/extensively drug resistant mutations in Mycobacterium tuberculosis isolated from the Tshepong National Health Laboratory Service Referral Tuberculosis Laboratory in North West.) to the review panel on the ( 29/04/2024).

The student ( Moshidisi), have responded and adjusted the protocol to the satisfactory level of the panel review committee and HES 3.1 was only signed after that.

The FRIC letter ( dated) severs as a confirmation of the CUT recognition of the project and route for a final screening and approval by the HSREC committee

Sincerely,



Prof P.M Makhoahle ( main supervisor) HPCSA no:MW010871
Associate Professor: Biomedical Technology
Faculty of Health and Environmental Sciences
Central University of Technology Free State

## Appendix G: Letter of permission from HOD

Central University of Technology, Free State

FACULTY OF HEALTH AND ENVIRONMENTAL SCIENCES

30 May 2024

**ATTN: UFS HSREC Ethics Committee**

**Re: Scientific Review; Motshidisi Jeanett Ntanjane**
**Topic: Sensitivity profiling of genes responsible for multi-/extensively drug resistant mutations in Mycobacterium tuberculosis isolated from the Tshepong National Health Laboratory Service Referral Tuberculosis Laboratory in North West.**

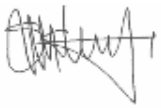
To Whom it may concern

This letter serves to confirm that the research protocol, titled, "***Sensitivity profiling of genes responsible for multi-/extensively drug resistant mutations in Mycobacterium tuberculosis isolated from the Tshepong National Health Laboratory Service Referral Tuberculosis Laboratory in North West***," has been reviewed the Faculty Research and Innovative Committee (FRIC) of the Faculty of Health and Environmental Sciences, Central University of Technology on the 23rd May 2024 and has been judged to be relevant, designed in accordance with accepted scientific practices and norms.

Student: Motshidisi Jeanett Ntanjane
Student number: 211058297

Should you require additional information, please contact Prof TJ Makhafola at jmakhafola@cut.ac.za

Sincerely;



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**Tel:** +27 051 507 3369 • **Fax:** +27 051 507 3369 • **E-mail:** jmakhafola@cut.ac.za • **Website:** www.cut.ac.za

## Appendix H: Proof of linguistics editing

**CORNELIA GELDENHUYS**

083 2877088
corrieg@mweb.co.za

24 September 2025

**TO WHOM IT MAY CONCERN**

Herewith I, **Cornelia Geldenhuys (ID 521114 0083 088)** declare that I am a qualified, accredited language practitioner and that I have edited the following dissertation:

**SENSITIVITY PROFILING OF GENES RESPONSIBLE FOR MULTI-/EXTENSIVELY DRUG RESISTANT MUTATIONS IN MYCOBACTERIUM TUBERCULOSIS ISOLATED FROM THE TSHEPONG NATIONAL HEALTH LABORATORY SERVICE REFERRAL TUBERCULOSIS LABORATORY IN NORTH-WEST**

**by**

**NTANJANE MOTSHIDISI JEANETT**

All changes were indicated by track changes and comments **for the author to verify, clarify aspects that are unclear, make the necessary adjustments, and finalise.** The editor takes no responsibility in the instance of this not being done. The document remains the final responsibility of the author.

.....
**C GELDENHUYS**
**MA (Lin) cum laude, MA (Mus), BA Hons (French), HED, HDL, UELM**

Accredited member/Geakkrediteerde lid, SATI, Membership/Lidmaatskap: 1001474 (A/E-E/A)
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## Appendix I: Turnitin Report- Digital receipt

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Sensitivity profiling of genes responsible for multi-/extensively drug resistant mutations in Mycobacterium tuberculosis isolated from the Tshepong National Health Laboratory Service Referral Tuberculosis Laboratory in North-West

by

NTANJANE MOTSHIDISI JEANETT

Thesis submitted for the fulfilment of the requirements for the Degree

MASTER OF HEALTH AND SCIENCES in

BIOMEDICAL TECHNOLOGY

in the

Department of Health Sciences
Faculty of Health and Environmental Sciences

of the

Central University of Technology, Free State
Bloemfontein South Africa

Supervisor: Prof P.M. Makhoahle (DHSc: Biomedical Technology, CUT)
Co-Supervisor: Prof S.S. Mashele (PhD: Dean: Faculty of Health and Environmental Sciences, CUT
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## Appendix J: Turnitin Report

Ntanjane Masters Thesis

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